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THE UNIVERSITY OF ALBERTA

ANIMAL MODEL STUDIES OF THERAPEUTIC TISSUE EXPANSION

by

JAMES GEORGE BEAUCHENE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

EXPERIMENTAL SURGERY

DEPARTMENT OF SURGERY

EDMONTON, ALBERTA

SPRING, 1988

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ISBN 0-315-42826-0

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled ANIMAL MODEL STUDIES OF THERAPEUTIC TISSUE EXPANSION submitted by James Beauchene in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE IN EXPERIMENTAL SURGERY.

.....
Supervisor

.....

.....

.....

Dated.....2/12/87.....

DEDICATION

To the injured children of this world - especially those for whom we, too often, still fall short as reconstructive surgeons.

A B S T R A C T

A small animal model for the study of dermal changes in response to therapeutic tissue expansion of the skin was developed and employed. The model involved the placement of a single Dow Corning silastic expansion device into the peritoneal cavity of the Sprague-Dawley rat.

A total of 118 animals were involved in this series of studies. In experimental animals the device was inflated with normal saline eight weeks after insertion. The expansion device in control animals remained uninflated. The animals were studied over 4, 8, 16, 32, and 64 days after expansion. Surface area changes were measured by a three dimensional surface scanning device,as were in vivo skin tensions measured by precision tensiometer. At the time of sacrifice, skin thickness was measured and skin specimens excised. Skin specimens were examined for breaking strength, collagen synthetic activity as measured by tritiated proline uptake, and total hydroxyproline content.

Results demonstrated that a two phase increase in surface area takes place with tissue expansion. Skin most centrally located over the expansion device contributes most to the increase in surface area with time. Skin tension increases immediately at the time of expander inflation but thereafter decreases towards normal. Skin thickness and

breaking strength were found to be initially decreased by the process of expansion but with time returned towards normal.

Collagen synthesis was demonstrated to be increased by the stress of therapeutic tissue expansion. Increased collagen synthesis resulted in a net accumulation of collagen as represented by increased total hydroxyproline content in skin subjected to tissue expansion.

ACKNOWLEDGEMENTS

I am indebted to a number of people for their special skills and advice. I acknowledge with gratitude the following:

Dr. Paul G. Scott - for his guidance, patience, and especially his remarkable understanding of clinical problems.

Dr. H.T.G. Williams - for his counsel in items non-scientific as well as scientific over several years and for specific advice regarding this set of investigations.

Miss M.M. Chambers - without whose toil at the biochemistry bench, much of this work would not have been possible.

Mrs. Trudy Hoogen - for her diligence and efficiency in working with small animals.

Mrs. Cheryl Souray - for doing the clerical work involved with this thesis and who managed to keep a sense of humour when all about her were losing theirs.

Dr. J.D.M. Alton - for his friendship over ten years and whose consideration in our office allowed me to work in the laboratory.

Miss Norine Falk - for her selflessness in assisting me in the laboratory when operations or specimens came due on weekends and evenings.

Dr. A. Peterson - for his time and dedication to the development of an engineering technique for mapping grid

points in animals in three dimensions.

Mr. Bob McLaren - for his persistence in the development and application of the mapping system and time spent assisting us after hours.

A. Pike and Associates - for their gift of Dow Corning expansion devices.

The Alberta Heritage Foundation for Medical Research and specifically Dr. Lionel McLeod - whose consideration and financial support allowed this research to occur.

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ANIMAL MODEL STUDIES OF THERAPEUTIC TISSUE EXPANSION

INTRODUCTION

A. Background

Reconstructive surgeons are challenged to repair or replace often formidable losses of soft tissue of the body, resulting in large areas of open wounds. The direct or indirect etiology of large areas of absence of tissue may be any one or a combination of: neoplasm, infection, congenital malformation, burn, metabolic disease, pressure, radiation, iatrogenic complication, or trauma. Of the tissues that must be reconstructed in such circumstances, the skin is usually the most critical. The integument provides an important immunological and mechanical barrier to infection in addition to providing a barrier to heat and water loss[1]. The skin also provides for thermoregulation and sensory functions. These functions are apart from the esthetic qualities of the integument which must also be taken into consideration by the reconstructive surgeon[2].

The abilities of all specialties of medicine are pushed to the limits of current technology in the treatment of patients. Patient expectations have increased[3], yet the health care systems of all countries are under increasing

scrutiny and financial pressure as a result of exponentially rising health care costs[4,5]. The surgeon is at the interface between these pressures and patient expectations.

There have been a number of advances in reconstructive surgery in the past fifteen years. Most notable have been the utilization of muscle based flaps[6] and the development of the technique of microsurgical free-tissue transfer[7]. They have allowed much more extensive skin defects to be reconstructed in a relatively expedient manner. These techniques however share the problems of other previously employed major reconstructive techniques[8]. These are: 1. donor site deficit (lack of physical ability as a result of loss of functioning muscle, insensibility, fibrous scarring, unsightly scarring), 2. risk of secondary complications such as hematoma or infection in a second operative site and 3. mismatch of donor to recipient site tissue (eg. thickness, texture, color, hair-bearing characteristics, and durability).

In addition, the techniques of muscle based transfer of tissue and microsurgical free tissue transfer have brought with them new problems. These are: 1) longer operative procedures with increased anaesthesia risk, 2) increased requirements of skills of surgeon and staff with the result that only selected highly trained personnel are appropriate

to perform such techniques, 3) increased demand on equipment and facility and resources of hospitals and 4) high financial costs.

The ideal technique of skin reconstruction would provide tissue with properties of texture, color, hair, durability and thickness identical to that of the skin lost[6]. Ideally it would also be simple and performed on an outpatient basis. Moreover, the ideal technique would not leave permanent sequelae at the donor site.

B. Literature Review

The concept of tissue expansion was first reported by Neumann in 1956 when he employed the progressive distention of an air filled rubber balloon implanted subcutaneously, in an attempt to reconstruct an ear[10]. The procedure was successful in providing sufficient skin to allow for satisfactory coverage of a costal cartilage graft auricular framework. Neumann only reported a single case in which he utilized tissue expansion. However, he pointed out the most obvious rewards of the concepts in "securing skin which would be high in quality in match of colour and texture with nearby skin, the avoidance of the need for a donor area in the usual sense of the term, and the possible reduction in the number of stages for a particular reconstructive effort".

The concept of physiologic tissue expansion is not new to man. The Ubangi tribe of Africa[11] and the Suya tribe of South America[12] have utilized the concept to attain lips and ear lobules of remarkable proportions for esthetic and cultural purposes for many centuries. Neither is the concept new to women who have been pregnant. Pregnancy leaves the soft tissues of the abdomen and skin in particular, redundant. It was this observation of the redundancy of the skin of his wife's abdomen after delivery that stimulated Radovan toward clinical application of the concept of tissue expansion[13]. Radovan is credited with the development and popularization of the technique[14]. Radovan presented his development and clinical experience with an inflatable silicone elastomer tissue expander in 1976[15]. In this paper he described its use primarily in reconstruction of the breast. He later published his experience with a large number of tissue expansion procedures in the reconstruction of defects of soft tissue in a wide variety of anatomic locations [16,17,18]. The low donor site expense , match of donor to recipient site tissue characteristics, simplicity and low financial cost were noted.

Argenta and Lapin reported their experience with clinical application of the technique in breast reconstruction and head and neck reconstruction[19,20,21].

In discussion of Radovan's paper , Grabb commented favorably on his own limited experience with the technique[22]. Grabb, a pre-eminent investigator in reconstructive surgery, stated, "Although many innovations are advanced, only a few have had the impact of microsurgery techniques and of muscle flaps. I predict that over the next 5 years , the technique of tissue expansion will be of equal importance."

The popularity of the technique for skin reconstruction has grown particularly in the last five years[23]. This has been in spite of the major criticism that the biologic basis of the phenomenon is poorly understood. In particular, the major question that has been raised is whether or not the tissue, during the course of therapeutic expansion, is merely stretched, or whether new tissue is generated - i.e. whether the tissue obtained is simply a "loan" or truly a "dividend" of valuable tissue[24].

Skin has been referred to by bioengineers as an "anisotropic elastic membrane"[25]. Prima facie it would appear that the skin overlying an inflated therapeutic tissue expansion device might respond simply as an elastic or semi-elastic material by stretching and thinning. This would result in a clear end point to the process- i.e.

rupture of the overlying skin or material. In a clinical setting this would be unsatisfactory, and in the least, present the surgeon and patient with possibly unsatisfactory skin tissue that has been stressed. The end result would be reconstructive tissue lacking in the desired qualities - particularly those of thickness, strength and durability. Further, once the tissue expansion device is removed and the defect repaired with the expanded tissue, were the skin to behave simply as an elastic material, one might expect a biomechanical recoil of the tissue. However, we know that although skin does behave as a semi-elastic material in the short term[26], in the long term it is a living tissue in a constant dynamic state with the capacity to repair and remodel[23,27].

Billingham and Medawar observed in 1955 that dermis adjacent to a contracting wound thinned in response to tensile stress, but later appeared to regain its premorbid thickness[28]. They described the process as "intussusceptive growth" . Abercrombie and James attempted to quantify these changes in the dermal collagen adjacent to the contracting wound by estimating hydroxyproline content[29]. Their results suggested that the amount of dermal hydroxyproline increases in scar tissue long after wounding, when healing appeared to be complete. Black et al. more recently measured skin thickness and collagen

content in obese patients and concluded that subcutaneous stretching induced by prolonged obesity lead to hypertrophy of collagen and that the skin had maintained or regained both its thickness and collagen content[30]. They also stated that it is unknown whether this is due to enhanced synthesis or decreased degradation.

Austad studied the dermis of expanded versus non-expanded skin flaps in guinea pigs and concluded that the skin of expanded flaps in pigs was thinner than that of non-expanded flaps[31]. Examination of his data suggests that the flaps had been greatly increased in length and width by tissue expansion. However since Austad's paper provided no absolute measurement of increase in surface area, neither the volume nor mass of the dermis could be extrapolated. In light of the earlier work by Billingham et al.[28], Abercrombie et al.[29] and Black et al.[30], we might have anticipated an absolute increase in volume and/or mass of expanded cutaneous tissue.

The suggestion that collagen synthesis specifically might be stimulated in dermis under tension is supported by the results of Yen and Suga who demonstrated such an effect in orthopedically stressed cranial sutures in mice[32]. The effects of stress in the synthesis and remodelling of connective tissue of bone and bone formation are well

known[33,34,35].

There is evidence that the epidermis in skin stretched by obesity[30] and by external devices experimentally does not thin, but in fact, proliferates towards maintaining a normal or greater epidermal thickness[36,37,38].

Cherry et al.[39] reported that expanded skin flaps in pigs behave from a vascular standpoint similar to delayed skin flaps[1], by developing an augmented vascular network. He demonstrated augmented vasculature histologically and angiographically. An increased unipedicle flap length survival in expanded and delayed flaps compared to control skin flaps was also demonstrated. This response in therapeutically expanded tissue may enhance the ability of dermis to generate new connective tissue. Both of these may in turn also suggest an increased resistance of slowly expanded skin to erosion by the expander in clinical circumstances.

Vander Kolk et al.[41] recently published a report on skin thickness, collagen content and collagen typing in expanded skin of buttock island flaps in pigs after ten days of expansion. Tissue sampling was performed by fixed

[1]The delay phenomenon is one wherein the vascularity and hence maximum surviving length of a reconstructive skin flap can be increased by prior surgical incision or manipulation(40).

cross-sectional area punch biopsy after expansion had occurred in the experimental group. Tissue sampling based on a post-expansion biopsy area yields no useful information with respect to quantitative changes. The area to be studied must be determined on a basis wherein the biopsy areas are identical prior to expansion. Only then can conclusions be drawn regarding changes that occur with expansion. Despite this, Vander Kolk et al. reported a 19% increase in collagen by dry weight, in the skin of expanded animals relative to controls, but that there was no difference in dry weights of specimens from the two groups. For collagen content to increase while overall dry weight remains the same, it would appear that another form or forms of tissues would have to be reduced and replaced by the collagen. This was not addressed in their discussion. They arrived at total skin collagen content by the addition of acid soluble content, pepsin soluble content and residue. These latter three figures were derived from two completely separate biochemical analyses and two different portions of a specimen. A method which involves the addition of three figures from two separate biochemical analyses on two separate samples is suspect conceptually and statistically. In addition to a 19% increase in collagen they reported a 17% decrease in sample thickness with expansion, despite the dry weights of specimens in each group being the same. Their measurements of expanded skin thickness were performed

on punch biopsy specimens prone to changes in dimension as a result of fixation. They also reported a relative increase in Type V collagen and a slight but not statistically significant decrease in content of Type III collagen in the expanded skin.

C. Objectives of Present Studies

The objectives of the studies described herein were to gain an understanding of the response of dermis during therapeutic tissue expansion. Specifically, these studies were directed to examination of collagen synthesis and collagen content in the skin of rats in response to tissue expansion. In order to correlate any changes that might be observed in collagen synthesis and collagen content of the dermis, this work also set out to examine material breaking strength of the skin tissue studied. For further correlative purposes we sought in this series of investigations to examine changes in surface area , skin thickness and skin tension with time, during the course of therapeutic tissue expansion.

The studies were divided into two projects. Project A was a pilot study designed to examine material breaking strength and collagen synthesis in response to tissue expansion in the skin of cohort rats. Project B was more extensive and designed to examine changes in surface area,

skin tension, skin thickness and total hydroxyproline content changes in response to tissue expansion in the skin of a comparable group of cohort rats.

MATERIALS AND METHODS

A. Project A

A total of 70 female Sprague-Dawley rats of the same birthdate and from the same supplier were obtained. Once the animals were received they were caged individually and observed for a minimum period of two weeks. The average body weight at the time of initial surgery was 218 ± 6 gm(mean ± s.e.m.). Prior to and following surgery, the animals were maintained two to a cage. The rats received a diet of Wayne Lab-Blox pellets (Allied Mills, Chicago Illinois) and water ad libitum. Environmentally the animals were exposed to 100% room air and normal ventilation. Ventilation allowed for fifteen air changes per hour which is considered a requirement of metabolically active animals. The room temperature was maintained at 20 + ^{o}C. The vivarium had no external windows and timed fluorescent lighting was provided for twelve hours each day. Relative humidity was maintained at 40-60%.

At age 10 weeks each animal underwent the following operation under methoxyflurane inhalational anaesthetic. Using sterile surgical technique, a single 50 mL Dow Corning tissue expansion device with self-contained valve was inserted through a midline laparotomy incision, into the

peritoneal cavity (PLATE 1). The device was fixed by each of its four tabs to the peritoneal surface of the abdominal wall with 4/0 nylon simple sutures to prevent rotation. The peritoneum and abdominal musculature were then closed with a single running simple 4/0 absorbable polyglycolic acid suture. The skin was reapproximated in a single layer with a running subcuticular suture of 4/0 polyglycolic acid with the knots buried.

Eight weeks after operation, the animals were randomly assigned to one of the five study time groups (4,8,16,32 and 64 days post-expansion). Experimental animals underwent percutaneous inflation of the intra-abdominal expansion device with a total of 120 mL of normal saline. Control animals underwent sham inflation with insertion of a 23 gauge needle into the valve of the expansion device and removal without injection. These and all subsequent procedures on experimental and control animals were performed under methoxyflurane general inhalational anaesthetic and with sterile technique in instrumentation and procedure.

B. Determinations in Project A

Sixteen hours prior to sacrifice, each animal underwent a minor surgical procedure under general anaesthesia in which an oblique incision was made across the left inguinal

PLATE 1. Examples of inflated and uninflated Dow-Corning tissue expansion devices. A 20 mL syringe with 23 ga needle is shown inserted into the inflated device.

area. Under 4.5 power magnification with loupes the soft tissues were dissected and the left iliac vein identified. Through a #30 gauge needle inserted into the left iliac vein, 0.20 mL of tritiated proline in saline solution (500 μCi per 1.0 mL) were injected under direct vision. Pressure was applied to the iliac vein for several minutes to prevent leakage and the skin wound was closed with Michel clips.

At each of the designated time periods of 4,8,16,32 and 64 days after expansion the animals were weighed, and tissue specimens were harvested in the following manner. Under general anaesthesia, two longitudinally oriented 3 x 1 cm strips of skin over the apex of the expansion device on the anterior abdomen were harvested. The skin strips excluded 5 mm of tissue on either side of the laparotomy incisional scar. The dimensions of the strips of abdominal skin were measured to an accuracy of ± mm and marked with a skin marking pen. The skin strips were excised with a # 20 scalpel blade. Specimens were placed in Parafilm™ wrapping and labelled. Intra-peritoneal incision was then made. The expansion device was retrieved and weighed intact. Using a syringe and 23 gauge needle, 1.5 mL of venous blood was withdrawn from the inferior vena cava. The animal was then allowed to expire under an overdose of inhalational anaesthetic.

One lateral skin specimen from each rat was subjected to breaking strength measurement in a custom-built extensiometer attached to a strain gauge (GRASS Force Displacement Transducer Model FT10C). Specimens were individually extended at a rate of 1.2 cm per minute with strain gauge output attached to a pen chart recorder (Fisher 5000). Load at failure was recorded from the peak of the stress/strain curve[42].

The remaining lateral skin specimen was stored frozen and underwent biochemical extraction as described by Scott et al.[43]. Specimens were weighed ,lyophilized and re-weighed (dry weight). One mL of 6N HCl was added to the sample which was then hydrolyzed for twenty hours at 105°C. The solution was dried on a Buchler Evapo-mix and the residue redissolved in 1 mL of 2M HCl. Proline was separated from hydroxyproline by passing a 0.5 mL sample of hydrolysate from the extraction procedure through an AG50W-X8 column, by the method of Schneir et al.[44]. Concentration of hydroxyproline was then determined colorimetrically by the method of Bergman et al. as follows[45]. The sample was made up to 500 mL with distilled H_2O. A 7 % W/V aqueous solution of Chloramine T in an acetate/citrate buffer (0.5 mL) was mixed with the sample and four minutes later 1 mL of Ehrlich's reagent solution was added. This solution was placed in a water

bath at 60°C for 21 minutes and then allowed to stand at room temperature for 60 minutes. Spectrophotometry readings were then performed at 562nm on a Gilford 250 spectrophotometer. The optical density of the samples was compared to standard hydroxyproline solutions and the amount of hydroxyproline per sample was calculated.

Samples of venous blood (1.5 mL) withdrawn from each animal, were placed in individual clotting tubes and analyzed for blood urea nitrogen in the Department of Biochemistry at the Surgical Medical Research Institute of the University of Alberta.

C. Project B

Forty-eight female Sprague-Dawley rats of identical birth date and supplier were obtained. At age 10 weeks each animal underwent surgical insertion of a single 50 mL Dow Corning tissue expansion device into the peritoneal cavity under general anaesthesia as in Project A. These and all subsequent procedures on experimental and control animals were performed under methoxyflurane inhalational anaesthetic and with sterile technique.

Eight weeks after insertion of the expansion device, each animal was randomly assigned to one of four study time

groups (4,8,16, and 32 weeks after expansion). A grid measuring 5 x 5 cm was tattooed on to the abdomen of each animal (PLATE 2). The grid consisted of 36 squarely placed dots each 1 cm apart, tattooed intradermally with India ink using an electrically-driven needle tattoo instrument. The upper border of the grid was oriented transversely along the costal margins bilaterally.

Each animal was then randomly assigned to either an experimental or control group for each time period. Experimental animals underwent percutaneous inflation of the expansion device with 120 mL of normal saline through a #23 gauge needle. Similarly, control animals underwent passage of the needle percutaneously into the valve of the expansion device without injection of saline.

In vivo skin tension measurements were performed with a precision tensiometer device designed and provided by Dr. J. Tyler of the Biomedical Design Centre of the University of Alberta (PLATE 3). The device consisted of two curved cutting surgical needles fixed on each side of the slide-type caliper of low mechanical resistance. Between the caliper was a fine instrument-grade mechanical spring with known and fixed load/displacement ratio. A miniature DC displacement transducer with digital readout (tolerance 0.0001 mm) was built into the device. The transducer

PLATE 2. Tattooed grid on animal abdomen

PLATE 3. Precision skin tensiometer device for indirect measurement of skin tension in vivo

measured displacement of the caliper.

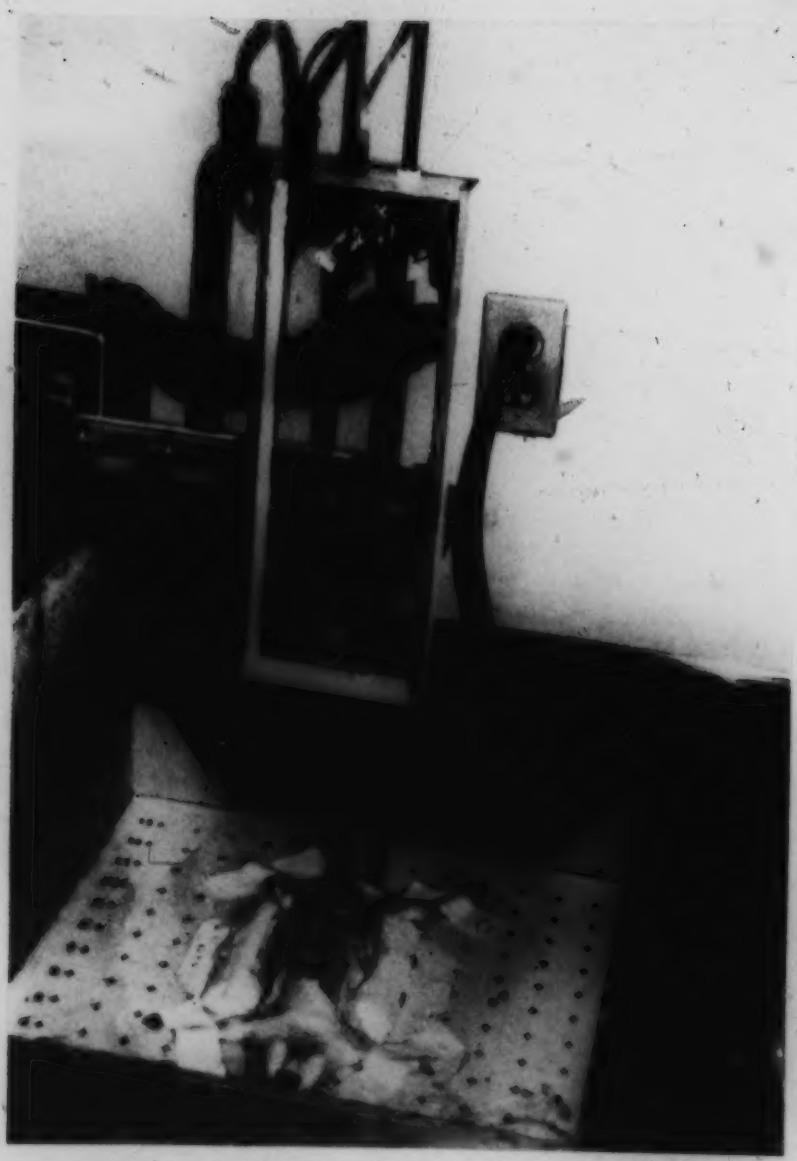
The use of the tensiometer device involved the placement of the surgical needles in a curvilinear manner into the skin boundaries of each of the right lateral, middle and left lateral tattooed grid strips. The needles of the tensiometer were fixed at a distance of 1 cm apart with the instrument compressed and the transducer calibrated to 0. The tensiometer was then allowed to relax and stabilize for 2 seconds and the digital reading of the strain gauge recorded. This was performed three times for each skin strip area and the average value recorded. Calibration of the device revealed that the device in full compression provided a load of 112 gm. Spring constant K in the device was essentially linear over the full range of the instrument. A load displacement ratio of 14 gm per mm was found for the instrument. Skin tension in gm was then calculated by the formula: skin tension = 112-(d x 14) where d= average displacement reading of the tensiometer.

Although the instrument is referred to herein, and by its inventor as a precision skin tensiometer, it measures skin tension indirectly. The device in fact measures displacement of resting skin <u>in vivo</u> resulting from a fixed linear tensile force. In addition to inherent skin tension, the amount of displacement measured is a function of the

stress/strain characteristics of the skin. Hence, the measurement of displacement with fixed force could also be a function of time. Time was held constant at two seconds of loading for each observation. We attempted to hold the stress/strain characteristics of the skin constant by examination of the same anatomic location of skin site on each animal by reference to the tattooed grid. Difficulties in measurement of inherent skin tensions in vivo are recognized in the literature [46].

In an attempt to gain an insight into the manner in which the skin stressed by the process of therapeutic tissue expansion changes in surface area and surface area configuration, Dr. A. Peterson of the Department of Civil Engineering , Faculty of Engineering, at the University of Alberta was consulted (Appendix A). An electrically driven surface scanning machine consisting of a highly sensitive light reflective probe attached to a series of stepping motors was developed and constructed by the Department of Civil Engineering (PLATE 4). During the process of scanning of the contour of the rat's abdomen with the animal fixed in a supine position under general anaesthesia, the light reflective probe maintained a constant distance of 2.54 cm from the surface from which light was reflected through a series of mechanical linkages and small electric motors with high power to weight ratios . The probe was

PLATE 4. Head and light-reflective probe of three dimensional surface scanner.



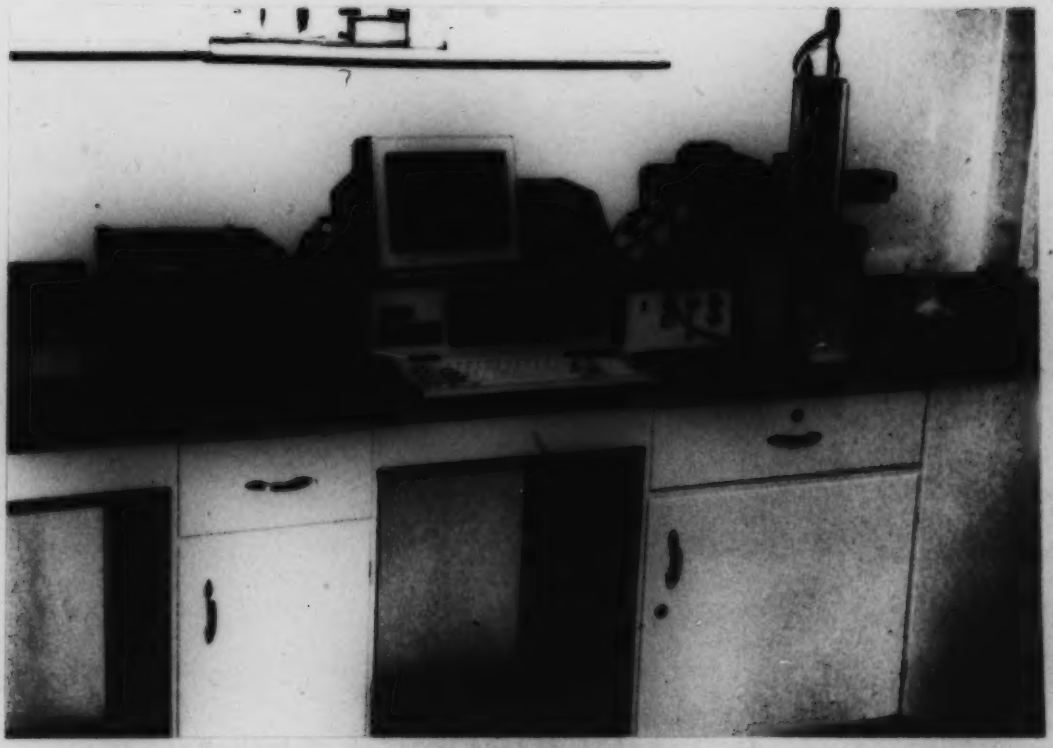
moved in the X and Y axes through stepping motors, and driven in a scan sequence by an IBM PC XT computer (PLATE 5). The computer was programmed to drive the probe back and forth across the entire width and length of the tattooed grid on the animal's anterior abdomen. Each transverse pass of the probe was 1 cm apart. The position of the probe tip in space during the scan was digitized for X,Y and Z axes and recorded by the same IBM PC XT computer. In addition, each of the tattooed grid points on the animal's anterior abdomen was manually targeted through movement of the probe controlled by a joystick,and driven through the computer. After targeting each of the tattooed grid points, the digital X , Y and Z axes digital information for each point was recorded by the computer. Each computer scan and recording of the tattooed grid points averaged 13 minutes. This was performed under general anaesthesia.

The animals in each of the four study time period groups underwent a total of five scans, equally spaced over time. At the time of each scan, body weight and skin tension were recorded.

D. Determinations in Project B

Sixteen hours prior to sacrifice, each animal received a single injection of 0.20 mL of tritiated proline in saline solution (500 μCi per 1.0 mL), into the iliac vein,

PLATE 5. Setup of computer-driven three dimensional digitizing surface scanner.



as in Project A. At the time of sacrifice, the animals were anaesthetized and weighed after removal of the Michel wound clips. The skin of the anterior abdomen over the tattooed grid site was clipped with a small animal hair clipper and shaved. At each of the inferior and superior poles of each of the lateral skin strips, a single transverse incision was made. A Kanon 0-25 mm micrometer caliper was used to measure skin thickness of the upper and lower poles of each of the lateral skin strips. The mean average measurement was recorded. Pressure applied to the skin during the course of skin thickness measurement was held constant at 100 ± 3 gm through the use of the micrometer ratchet device with click stops.

The general anaesthetic was continued in each case and using a #20 scalpel blade, the pre-determined skin strips defined by the pre-expansion grid tattoo were meticulously excised. The strips were individually wrapped in Parafilm^{TM} and labelled.

The breaking strength of one of the strips from each animal was determined as previously described. This was performed within a few hours of specimen harvesting during which time they were stored at 4^{o}C.

The remaining skin specimen was weighed and stored at

-20°C. This sample was later hydrolyzed and analyzed for total hydroxyproline and specific activity of hydroxyproline, as previously described for Project A.

E. Data Analysis

Data regarding mapping of the tattooed grid points in three dimensions were entered into and analyzed on the Amdahl 5860 main frame computer at the University of Alberta. All other data was entered into and analyzed on an IBM AT computer using Crisp statistics software. The Mann-Whitney statistical test for difference was chosen as the test of choice because of the relatively small numbers involved and the fact that this test, being non-parametric, makes no assumption about the sample distribution. The Mann-Whitney test is consequently considered to be a conservative test for statistical difference. The alpha level was set at 0.05.

III RESULTS

A. Project A

a. Body Weights

Examination of the body weights revealed a progressive increase in body weights of each of the experimental and control groups at each time period (TABLE 1). There were no significant differences in mean body weights between experimental and control groups for any of the times studied.

b. Weights of Expansion Devices

Examination of the weights of inflated and uninflated expansion devices retrieved after sacrifice revealed no evidence of leakage resulting in loss of inflation or evidence of fluid accumulation in uninflated expansion devices (TABLE 2).

c. Blood Urea Nitrogen

There were no statistically significant differences found between the blood urea nitrogens of the control and experimental groups at four, eight, sixteen and sixty-four

TABLE 1. Body Weights of Experimental and Control Animals in Project A.

Table 1

Body Weight[a]
(gm, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,c]	p value[d] (<)
0	271±5	273±5	12	0.75
4	271±5	273±5	12	0.57
	(p<0.81)	(p<0.94)		
0	273±5	276±5	13	0.46
8	278±5	282±5	13	0.60
	(p<0.35)	(p<0.46)		
0	278±7	273±8	12	0.75
16	286±7	282±7	12	0.75
	(p<0.42)	(p<0.39)		
0	270±6	272±4	10	0.60
32	287±6	290±5	10	0.75
	(p<0.14)	(p<0.03)		
0	264±7	280±8	11	0.32
64	287±8	305±9	11	0.20
	(p<0.05)	(p<0.04)		

a) adjusted for weight of expansion device

b) total of experimentals and controls in each time-group

c) a total of 12 animals were lost due to anaesthetic(7) and post-operative(5) deaths

d) by Mann-Whitney test

TABLE 2. Weights of Inflated and Uninflated Expansion Devices Retrieved

Table 2

Weight of Expander Retrieved
(gm, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,c]
4	138.5±0.2	18.5±0.2	12
8	138.4±0.4	18.4±0.2	13
16	138.3±0.3	18.6±0.2	12
32	137.9±0.4	18.5±0.3	10
64	137.4±0.2	18.5±0.3	11

b) total of experimentals and controls in each time-group

c) a total of 12 animals were lost due to anaesthetic(7) and post-operative(5) deaths

days (TABLE 3). A statistically significant difference was noted between the experimental and control blood urea nitrogens at 32 days (experimental 19.0 mg/dL, control 22.1 mg/dL). A low standard error of the mean was also noted in each of these two groups (experimental 0.6, control 0.7)

d. Breaking Strength

Results of testing of load to initial failure of the skin specimens revealed the breaking strengths of the experimental animals to be significantly decreased relative to controls at four and eight days (FIGURE 1). Mean breaking strengths of both the control and experimental animals at 16 days were lower than those of all the other time groups. However, there was no statistically significant difference between mean breaking strength of skin specimens of the experimental and control animals in the sixteen day group. Differences in mean breaking strength between experimental and control animals at thirty-two and sixty-four days were not found to be statistically significant.

e. Specific Activity

At four days after expansion, specific activity of tritiated hydroxyproline was found to be significantly elevated by a factor of approximately six fold in the skin

TABLE 3. Blood Urea Nitrogen Data for Experimental and Control Animals in Project A.

Table 3

Blood Urea Nitrogen
(mean mg/dl ± 1 s.e.m.)

Days	Experimental	Control	n[b,c]	p value[d] (<)
4	16.6±1.3	19.1±0.4	12	0.06
8	17.2±0.7	18.3±0.7	13	0.09
16	21.7±1.6	20.2±1.3	12	0.45
32	19.0±0.6	22.1±0.7	10	0.02
64	19.3±0.8	19.8±1.0	11	0.86

b) total of experimentals and controls in each time-group

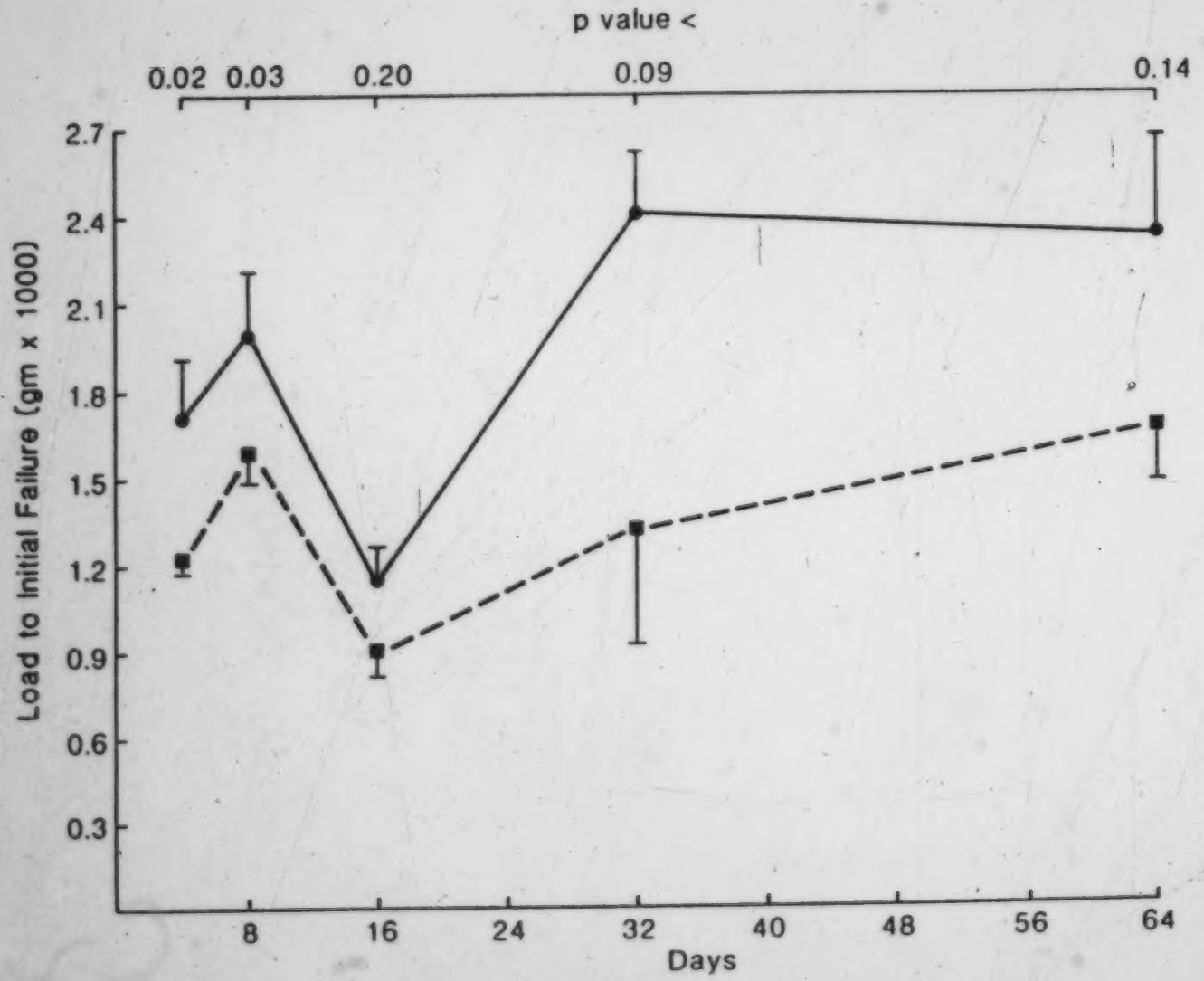
c) a total of 12 animals were lost due to anaesthetic(7) and post-operative(5) deaths

d) by Mann-Whitney test

FIGURE 1. A comparison of breaking strengths of skin specimens from experimental and control animals in Project A. Standard errors of the means are represented by bars. The results of statistical analysis, as described in the text, are shown at the top.

■ experimental animals

● control animals


p value <
0.02
0.03
0.20
0.09
0.14
Load to Initial Failure (gm x 1000)
2.7
2.4
2.1
1.8
1.5
1.2
0.9
0.6
0.3
8
16
24
32
40
48
56
64
Days

of the experimental animals relative to control animals. (FIGURE 2). Statistically significant elevations of specific activity in the skin of experimental animals were also found subsequently at days eight, and sixteen post-expansion. The difference in specific activity at thirty-two days post-expansion, was not significant, although the p value was <0.07. There was no difference in specific activity in the skin of control and experimental animals at sixty-four days after expansion.

B. Project B

a. Body Weight

Body weight of the four day experimental animals decreased from a mean of 2275 ± 8.3 gm to 249 ± 5.7 gm four days post-expansion (FIGURE 3). Similarly , the mean body weight of the control animals decreased from 267 ± 4.2 gm to 249 ± 5.2 gm four days post expansion.

The mean body weight of the 8 day experimental animals did not change significantly over eight days . Similarly there was no statistical difference in the body weights of the 8 day control animals in the 8 day time period (FIGURE 4).

FIGURE 2. A comparison of specific activity of ^{3}H hydroxyproline in skin specimens from experimental and control animals in Project A. Standard errors of the means are represented by bars.

■ experimental animals

● control animals

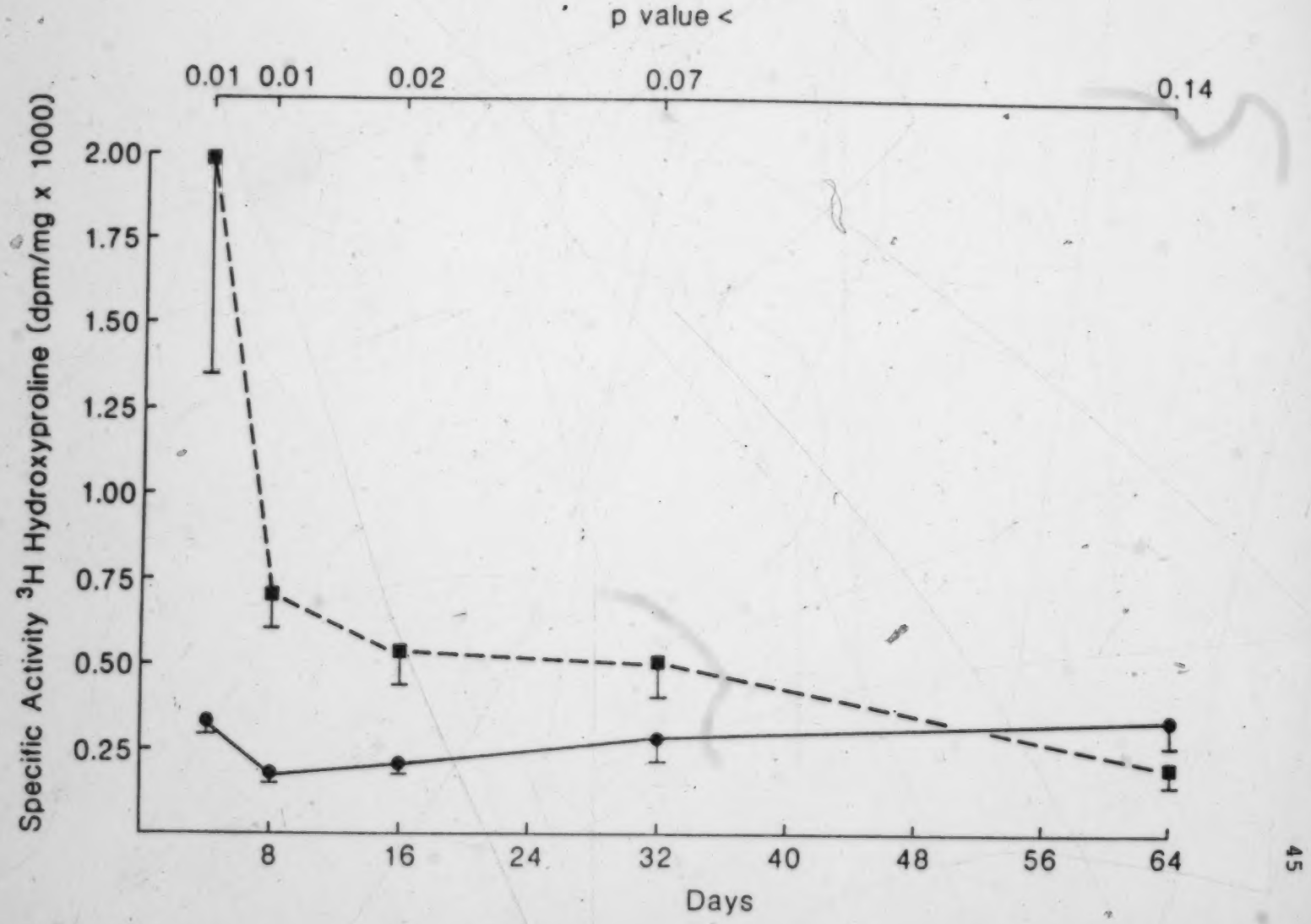

p value <
0.01 0.01 0.02 0.07 0.14
Specific Activity ^{3}H Hydroxyproline (dpm/mg x 1000)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
8 16 24 32 40 48 56 64
Days

FIGURE 3. A comparison of body weights (adjusted for weight of inflated or uninflated expanders) of experimental and control animals studied for 4 days in Project B. Standard errors of the means are represented by bars.

■ experimental animals

● control animals

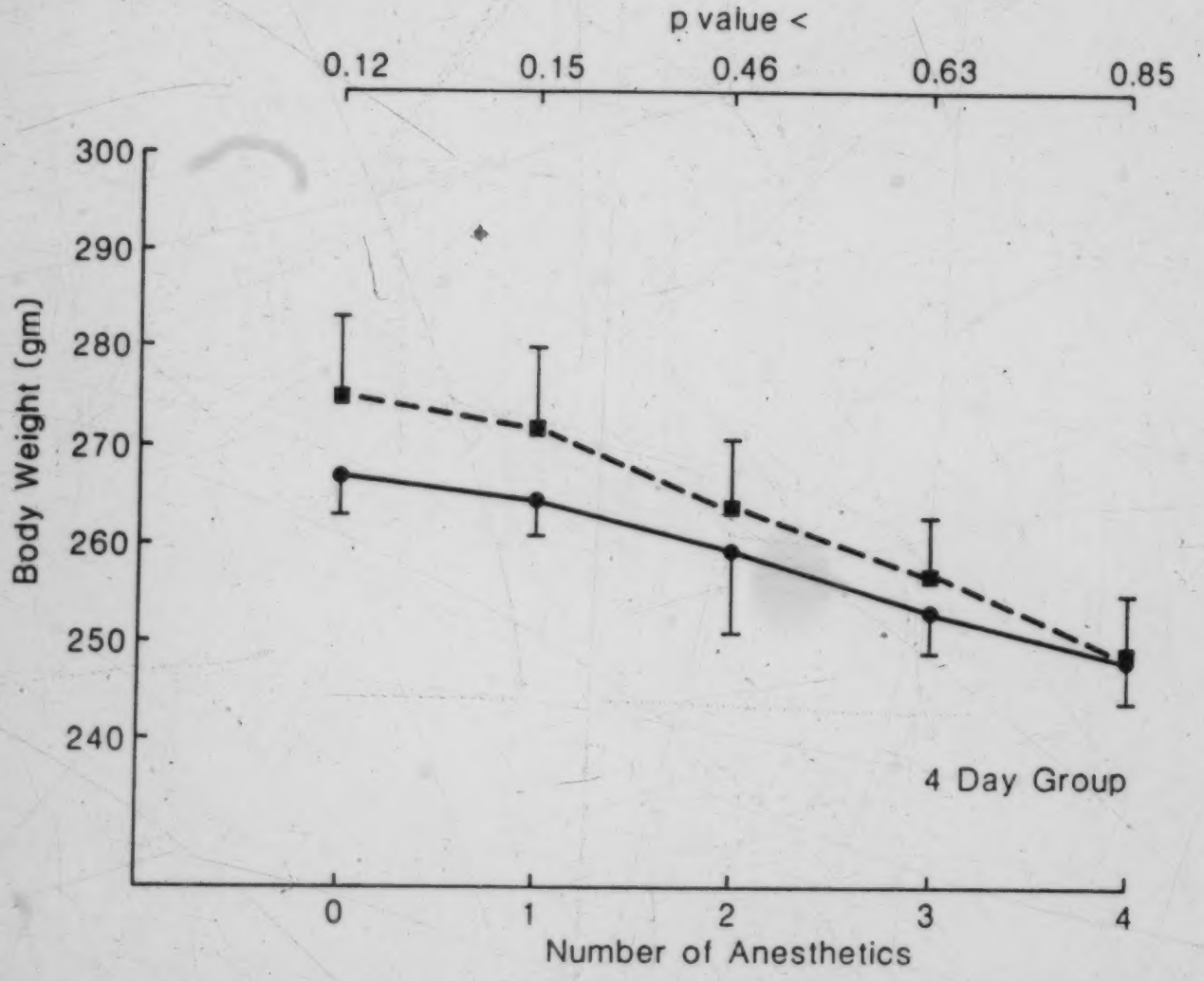
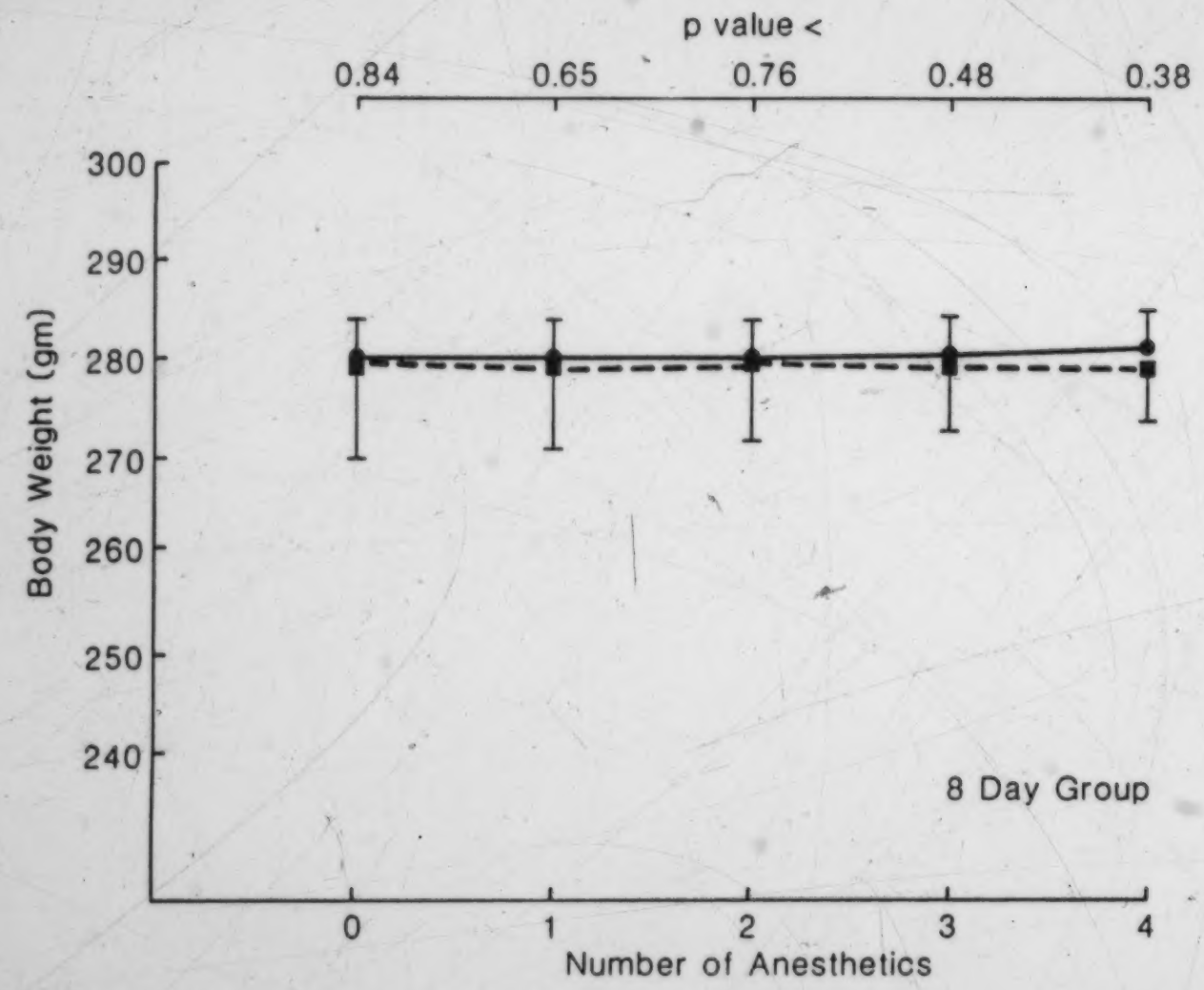

p value <
0.12
0.15
0.46
0.63
0.85
300
290
280
270
260
250
240
Body Weight (gm)
4 Day Group
0
1
2
3
4
Number of Anesthetics

FIGURE 4. A comparison of body weights (adjusted for weight of inflated or uninflated expanders) of experimental and control animals studied for 8 days in Project B. Standard errors of the means are represented by bars.

■ experimental animals

● control animals


p value <
0.84
0.65
0.76
0.48
0.38
300
290
280
270
260
250
240
Body Weight (gm)
8 Day Group
0
1
2
3
4
Number of Anesthetics

Mean body weights of the sixteen day experimental animals increased from 278 ± 9.8 gm to 286 ± 8.7 gm over sixteen days after expansion (FIGURE 5). The mean body weights of the sixteen day control animals increased from 276 ± 5.6 gm to 291 ± 5.5 gm. This represented an average increase of approximately 12 g for the two groups.

The 32 day experimental group mean body weight increased from 267 ± 4.6 gm to 280 ± 3.8 gm over thirty-two days (FIGURE 6). The mean body weight of the 32 day control animals increased from 265 ± 4.9 gm to ±294 gm. This represented an average increase of 19 gm over 32 days.

b. Surface Area Changes

In addition to the overall 5 x 5 cm tattoo grid surface area, several other component surface areas were analyzed (PLATE 6).

Surface areas of the entire 5 x 5 cm grid demonstrated less variance than those of the lateral 3 x 1 cm strips alone (Appendix C, Tables 9-16). Mean surface areas of the 5 x 5 cm grid for each scan and each study group were examined.

Surface area of the 5 x 5 cm grid in the four day

FIGURE 5. A comparison of body weights (adjusted for weight of inflated or uninflated expanders) of experimental and control animals studied for 16 days in Project B. Standard errors of the means are represented by bars.

■ experimental animals

● control animals

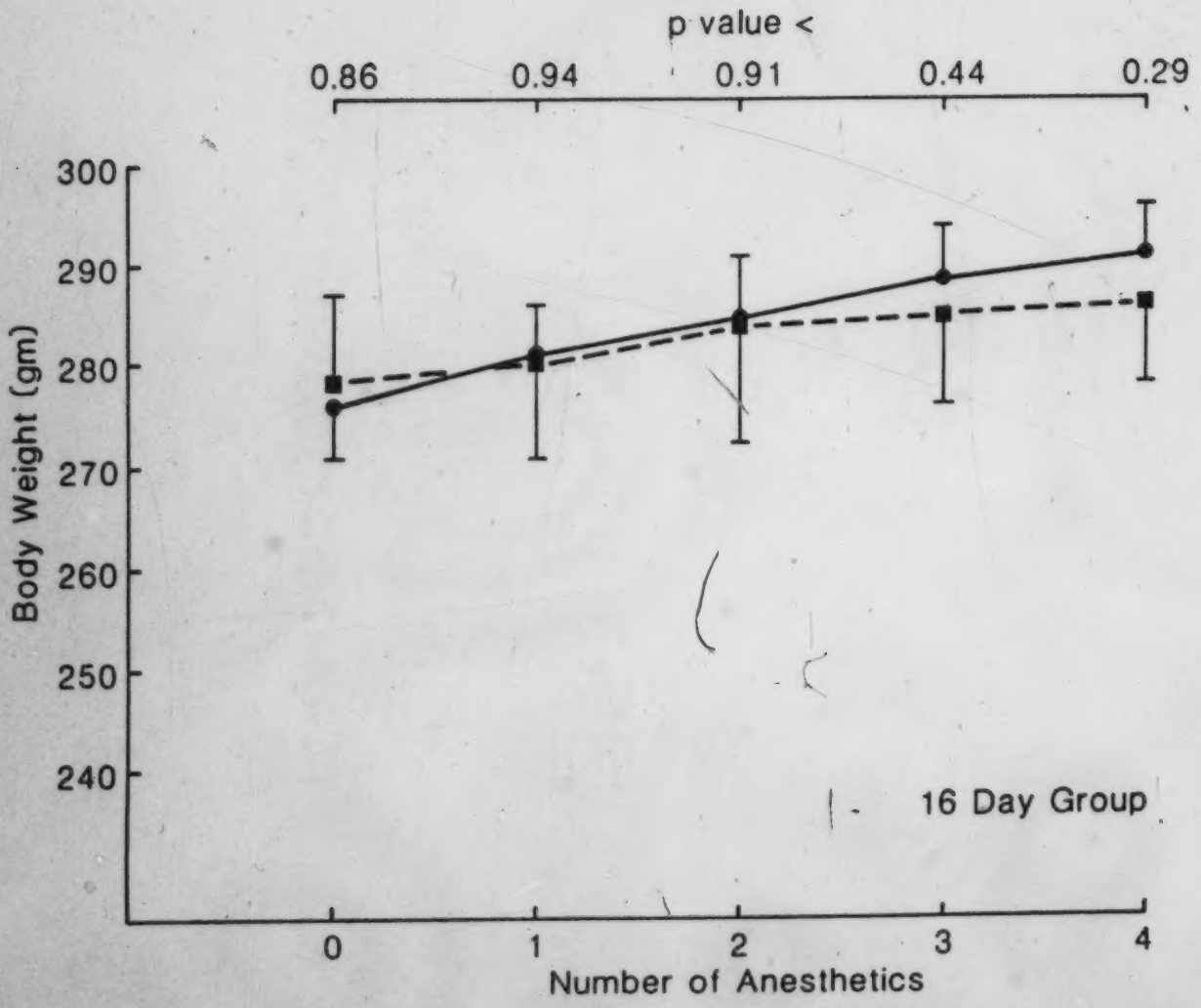

p value <
0.86
0.94
0.91
0.44
0.29
300
290
280
270
260
250
240
Body Weight (gm)
16 Day Group
0
1
2
3
4
Number of Anesthetics

FIGURE 6. A comparison of body weights (adjusted for weight of inflated or uninflated expanders) of experimental and control animals studied for 32 days in Project B. Standard errors of the means are represented by bars.

■ experimental animals

● control animals

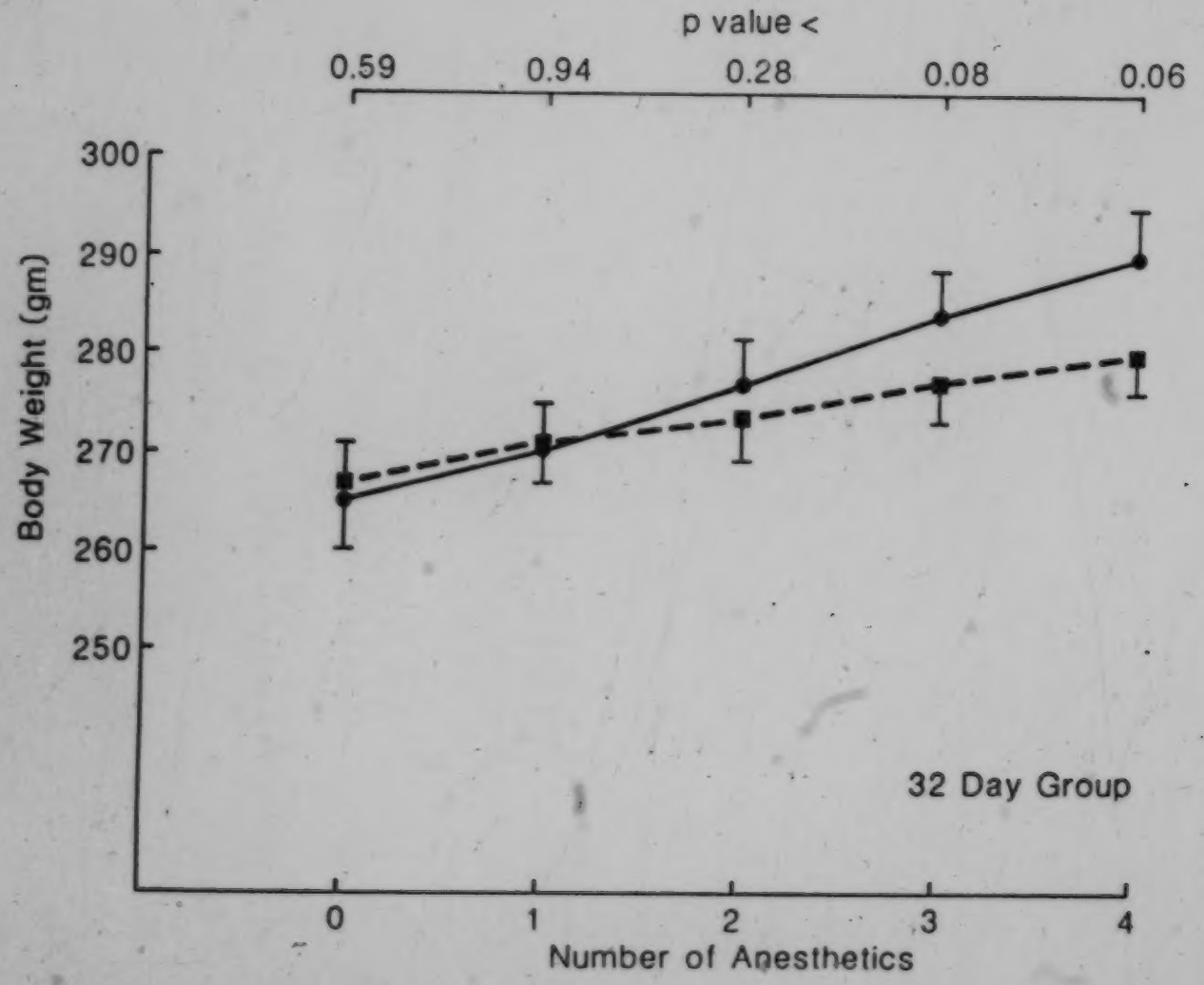
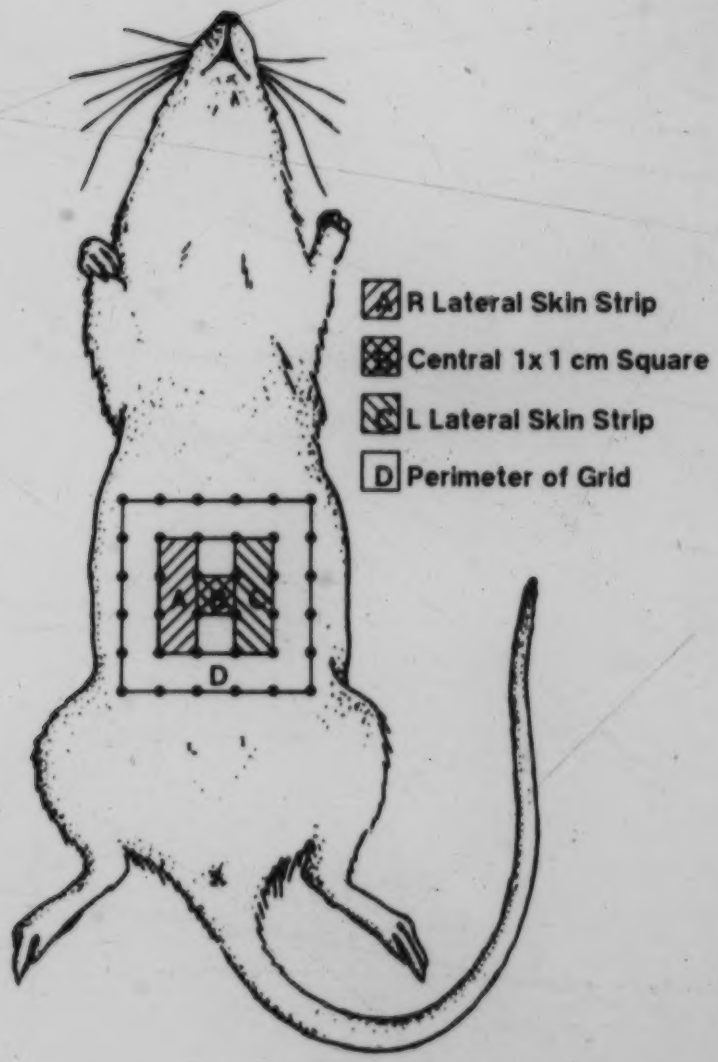

p value <
0.59
0.94
0.28
0.08
0.06
300
290
280
270
260
250
Body Weight (gm)
32 Day Group
0
1
2
3
4
Number of Anesthetics

PLATE 6. Artist's representation of surface areas scanned and analyzed.


R Lateral Skin Strip
Central 1x1 cm Square
L Lateral Skin Strip
D Perimeter of Grid
D

experimental group increased immediately at the time of expansion by 32.8 ± 1.9% (FIGURE 7). Over four days this remained approximately the same at 31 ± 4.4%. In the control animals over four days there appeared to be a small increase in the first two days and a small decrease over the last two days.

In the eight day experimental group the 5 x 5 grid surface area increased 35.5% at the time of inflation and demonstrated an increase to 141.5 ± 5.6% over the remaining eight days (FIGURE 8). The 8 day control animals' surface area changes showed an increase with time of approximately 10% ± 5.9%.

The experimental 16 day group exhibited a 29.5% increase in original 5 x 5 cm area immediately with inflation of the expander (FIGURE 9). This rose to only 143% + 9.9% over 16 days. Control animals in the 16 day group underwent a 15.8% increase in surface area of the original 5 x 5 cm grid over 16 days.

The 32 day experimental animals exhibited a 40% increase in surface area of the entire grid immediately after inflation of the expander and this rose to 162.4 ± 5.3% , 169.4 ± 6.7% and 171.6 ± 6.7% at 8,16,24 and 32 days after expansion, respectively (FIGURE 10). The same pre-expansion

FIGURE 7. A comparison of changes in surface areas of entire 5 x 5 cm grid in experimental and control animals studied daily for 4 days in Project B. Standard errors of the means are represented by bars. Surface area is expressed relative to original (unexpanded) area.

■ experimental animals

● control animals

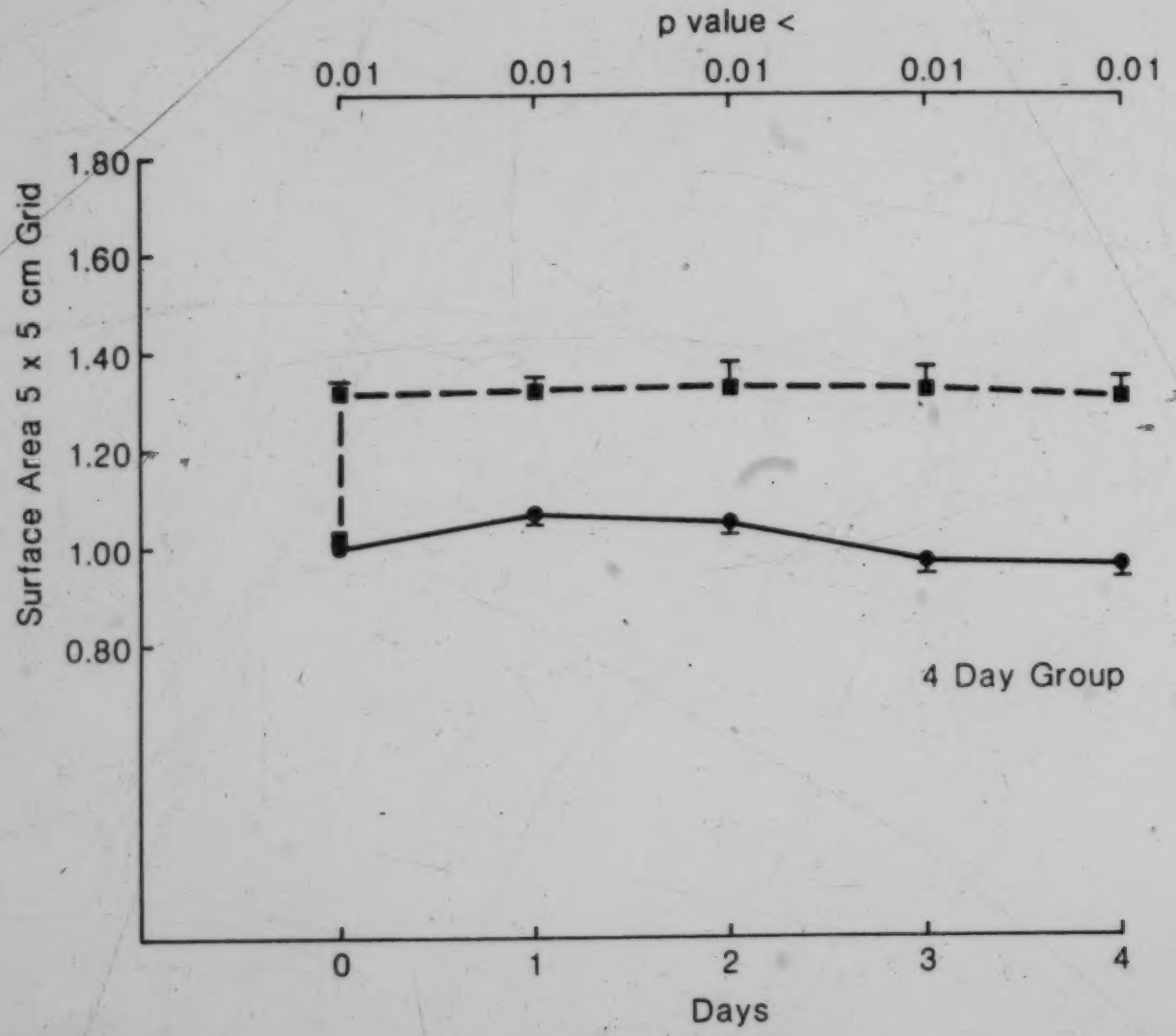

p value <
0.01
0.01
0.01
0.01
0.01
Surface Area 5 x 5 cm Grid
1.80
1.60
1.40
1.20
1.00
0.80
4 Day Group
0
1
2
3
4
Days

FIGURE 8. A comparison of changes in surface area of entire 5 x 5 cm grid in experimental and control animals studied for 8 days in Project B. Standard errors of the means are represented by bars.

■ experimental animals

● control animals

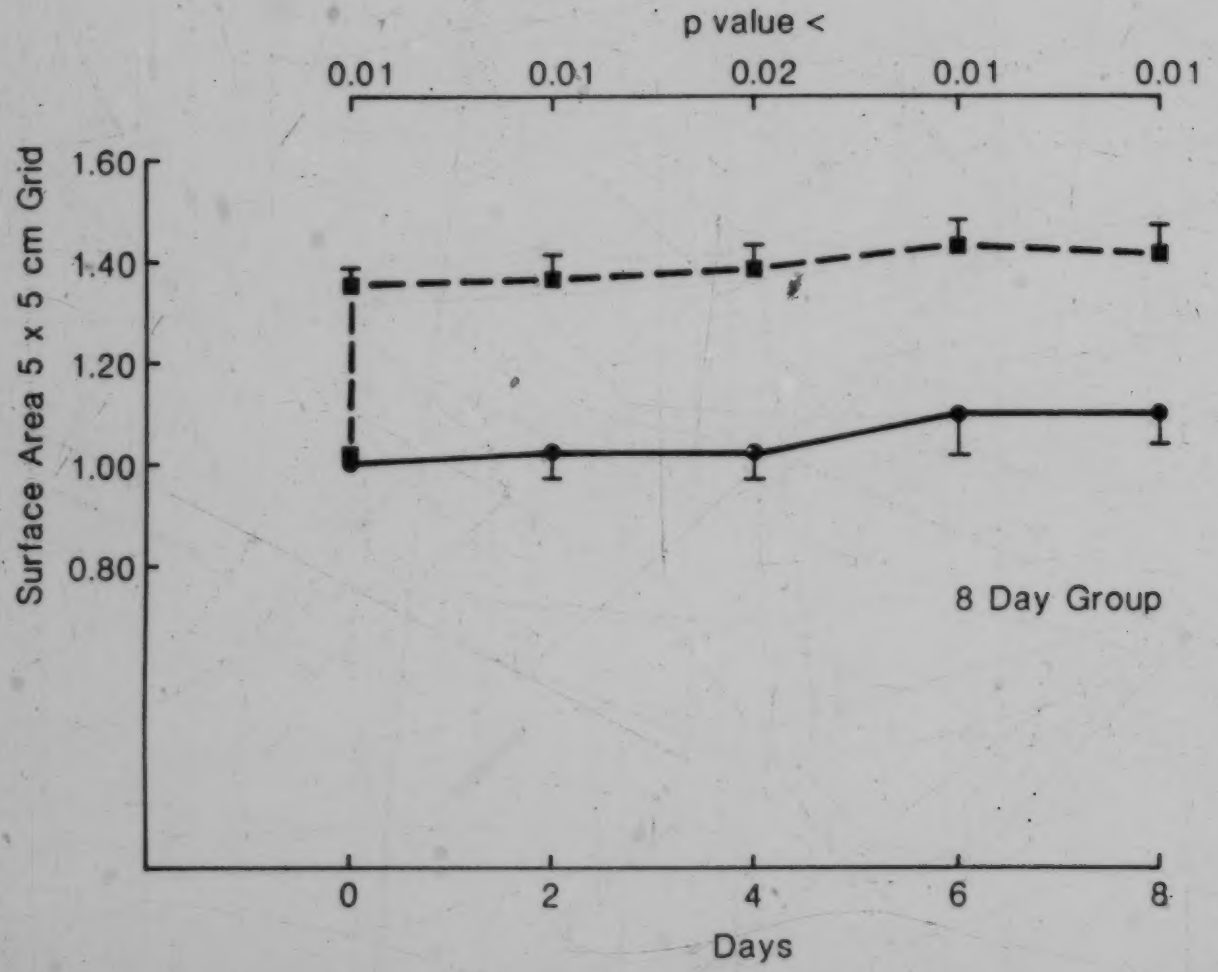

p value <
0.01
0.01
0.02
0.01
0.01
Surface Area 5 x 5 cm Grid
1.60
1.40
1.20
1.00
0.80
8 Day Group
0
2
4
6
8
Days

FIGURE 9. A comparison of changes in surface area of entire 5 x 5 cm grid in experimental and control animals studied for 16 days in Project B. Standard errors of the means are represented by bars.

■ experimental animals

● control animals

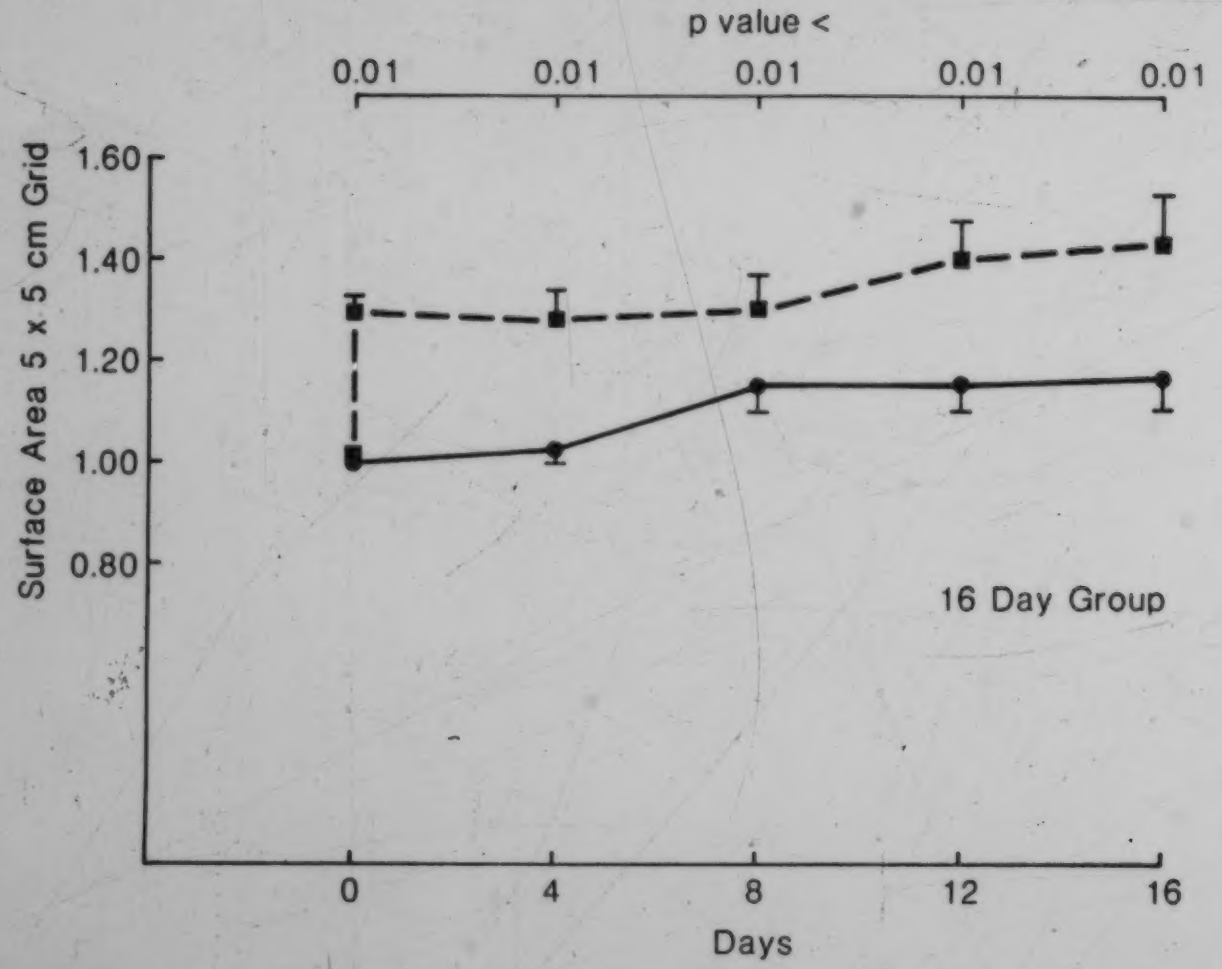
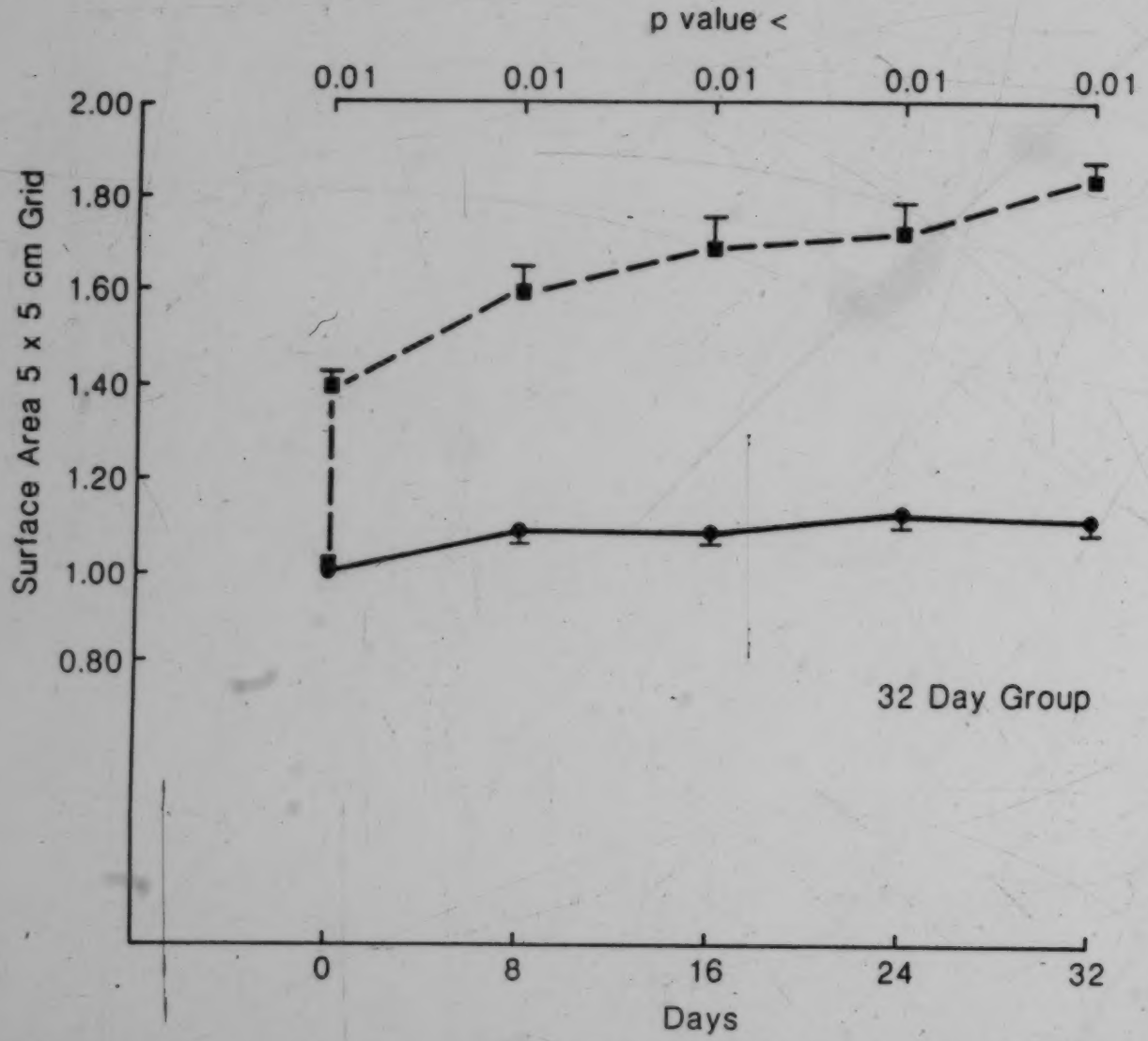

p value <
0.01
0.01
0.01
0.01
0.01
Surface Area 5 x 5 cm Grid
1.60
1.40
1.20
1.00
0.80
16 Day Group
0
4
8
12
16
Days

FIGURE 10. A comparison of changes in surface area of entire 5 x 5 cm grid in experimental and control animals studied for 32 days in Project B. Standard errors of the means are represented by bars.

■ experimental animals

● control animals


p value <
0.01
0.01
0.01
0.01
0.01
2.00
1.80
1.60
1.40
1.20
1.00
0.80
Surface Area 5 x 5 cm Grid
32 Day Group
0
8
16
24
32
Days

surface area in the control animals rose 16.4 ± 11.1% over 32 days.

The surface areas of the pre-expansion central 1 x 1 cm grid were compared to the surface area changes in the 1 cm perimeter of the original tattoo grid (PLATE 6). At the time of inflation similar increases in surface area were noted in the two groups. After four days of expansion, the central 1 x 1 cm grid had increased a further 12% in area while the perimeter decreased 5.0% (FIGURE 11). In the eight day group, the central 1 x 1 cm strip in the experimental animals increased a further 38.2% while the perimeter demonstrated a decrease of 3.9% (FIGURE 12). In the 16 day experimental group, the central 1 x 1 cm grid increased in area a further 29% while the perimeter increased in area 11.1% (FIGURE 13). In the 32 day experimental group, over 32 days post expansion, the central 1 x 1 cm grid increased a further 78.5% and the perimeter increased in surface area a further 36.9% (FIGURE 14).

c. Skin Tension

In the four day experimental group, skin tension rose from a mean of 67.5 g to 91.1 gm immediately after inflation of the expansion device (FIGURE 15). Over the remaining four days, the skin tension decreased in a curvilinear

FIGURE 11. A comparison of changes in surface area of central 1 x 1 cm square and mean surface area of perimeter 1 x 1 cm square in experimental animals studied daily in Project B. Standard errors of the means are represented by bars.

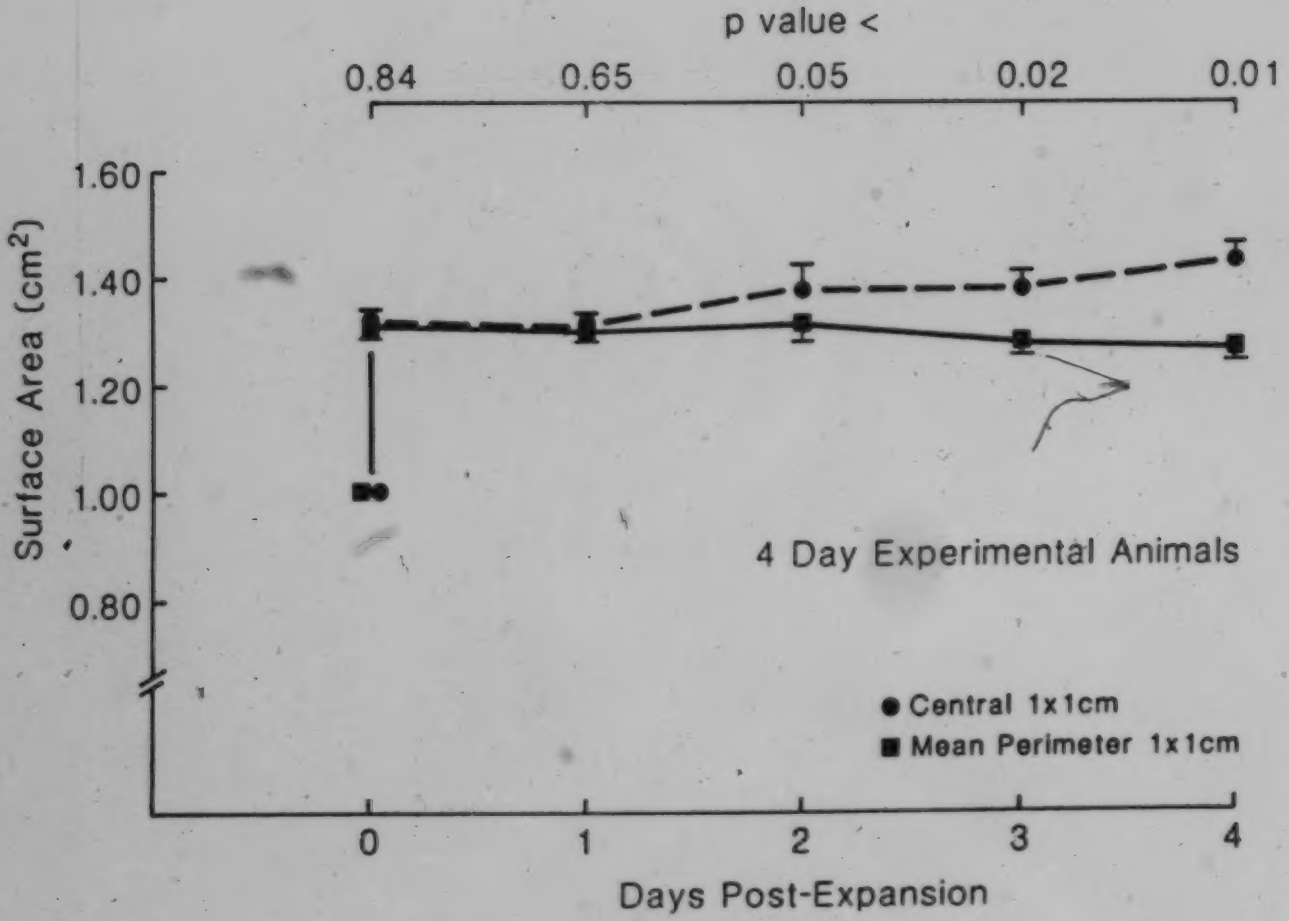

p value <
0.84
0.65
0.05
0.02
0.01
1.60
1.40
1.20
1.00
0.80
Surface Area (cm²)
4 Day Experimental Animals
● Central 1x1cm
■ Mean Perimeter 1x1cm
0
1
2
3
4
Days Post-Expansion

FIGURE 12. A comparison of changes in surface area of central 1 x 1 cm square and mean surface area of perimeter 1 x 1 cm square in experimental animals studied for 8 days in Project B. Standard errors of the means are represented by bars.

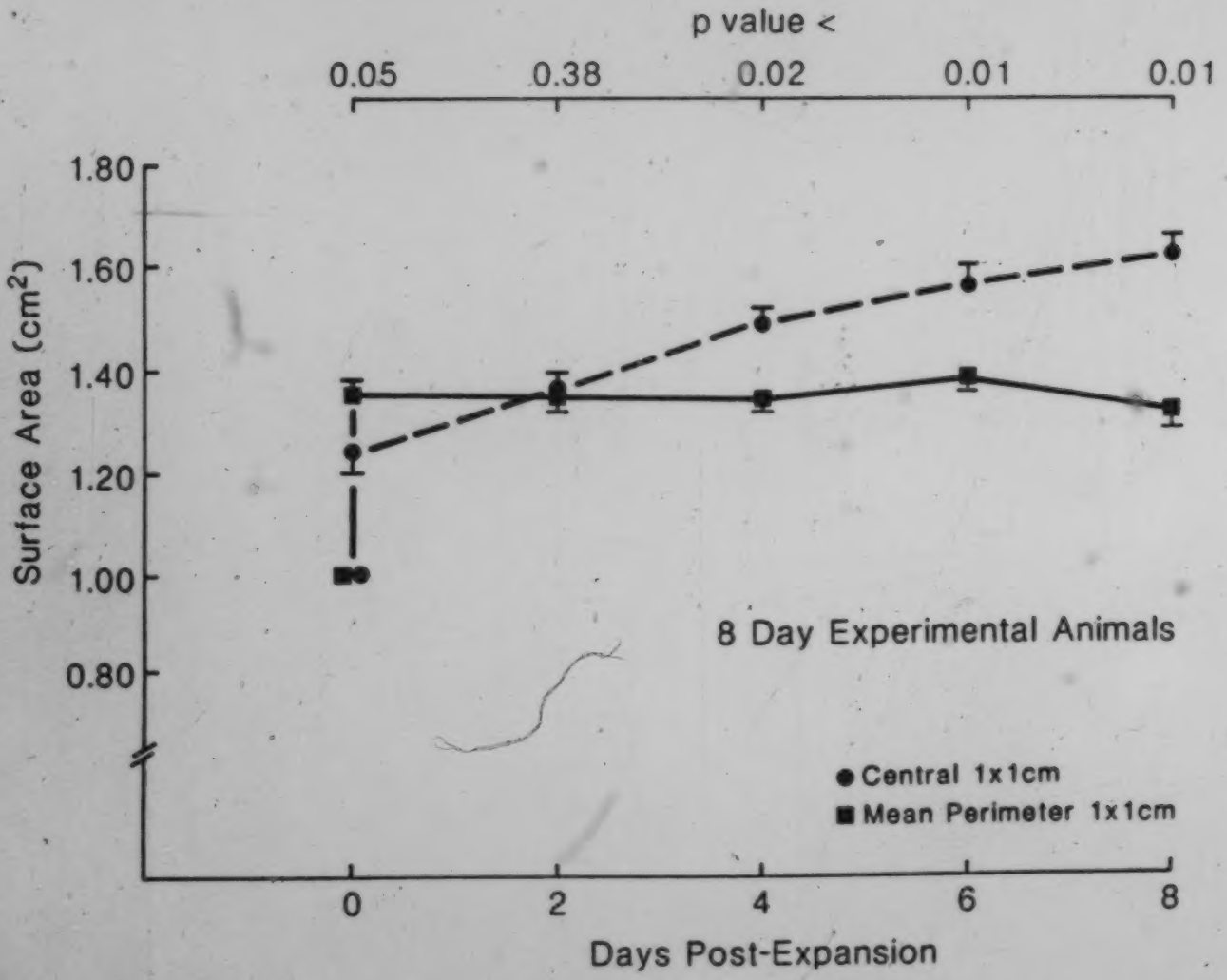

p value <
0.05
0.38
0.02
0.01
0.01
1.80
1.60
1.40
1.20
1.00
0.80
Surface Area (cm²)
8 Day Experimental Animals
● Central 1x1cm
■ Mean Perimeter 1x1cm
0
2
4
6
8
Days Post-Expansion

FIGURE 13. A comparison of changes in surface area of central 1 x 1 cm square and mean surface area of perimeter 1 x 1 cm square in experimental animals studied for 16 days in Project B. Standard errors of the means are represented by bars.

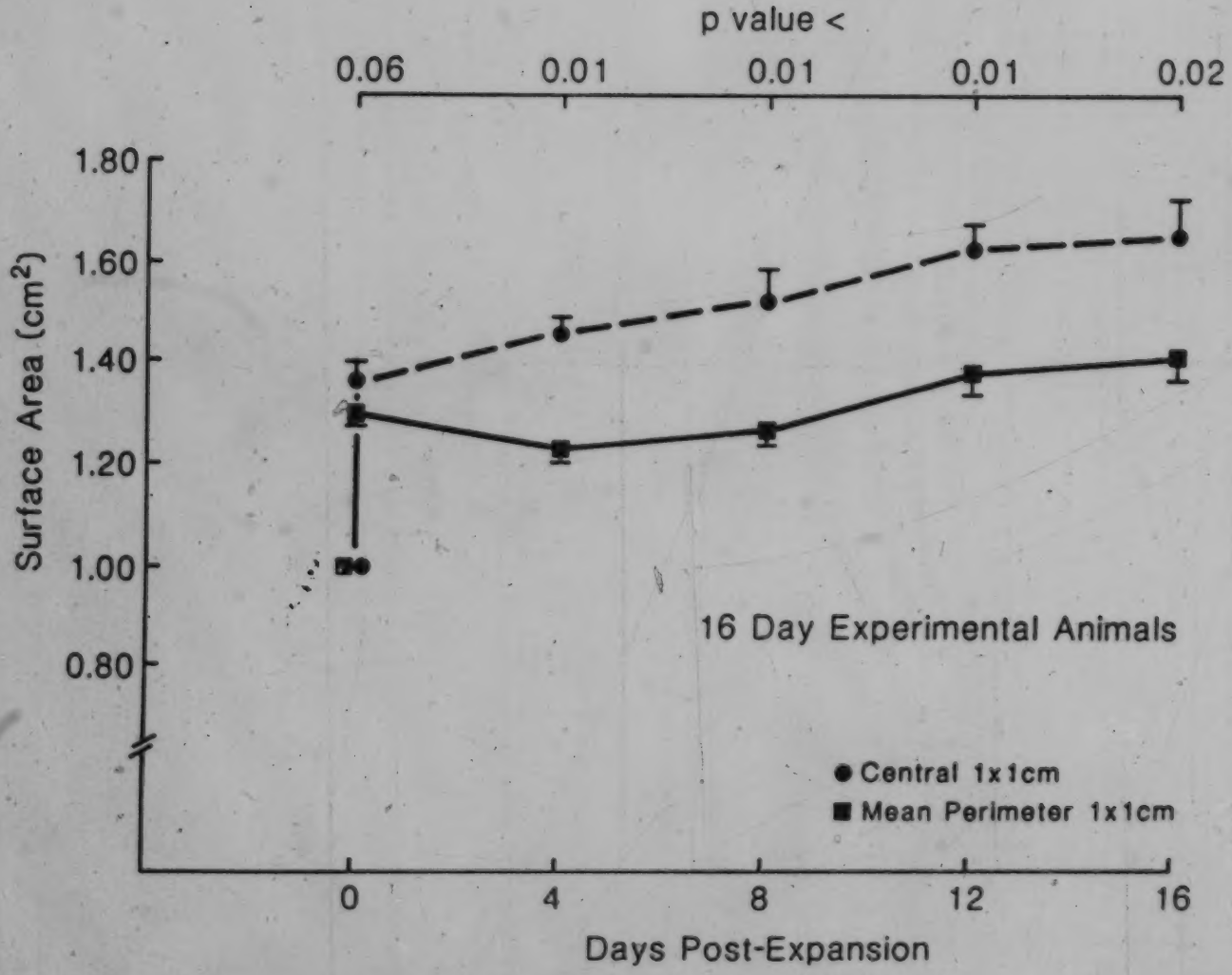

p value <
0.06
0.01
0.01
0.01
0.02
1.80
1.60
1.40
1.20
1.00
0.80
Surface Area (cm²)
16 Day Experimental Animals
● Central 1x1cm
■ Mean Perimeter 1x1cm
0
4
8
12
16
Days Post-Expansion

FIGURE 14. A comparison of changes in surface area of central 1 x 1 cm square and mean surface area of perimeter 1 x 1 cm square in experimental animals studied for 32 days in Project B. Standard errors of the means are represented by bars.

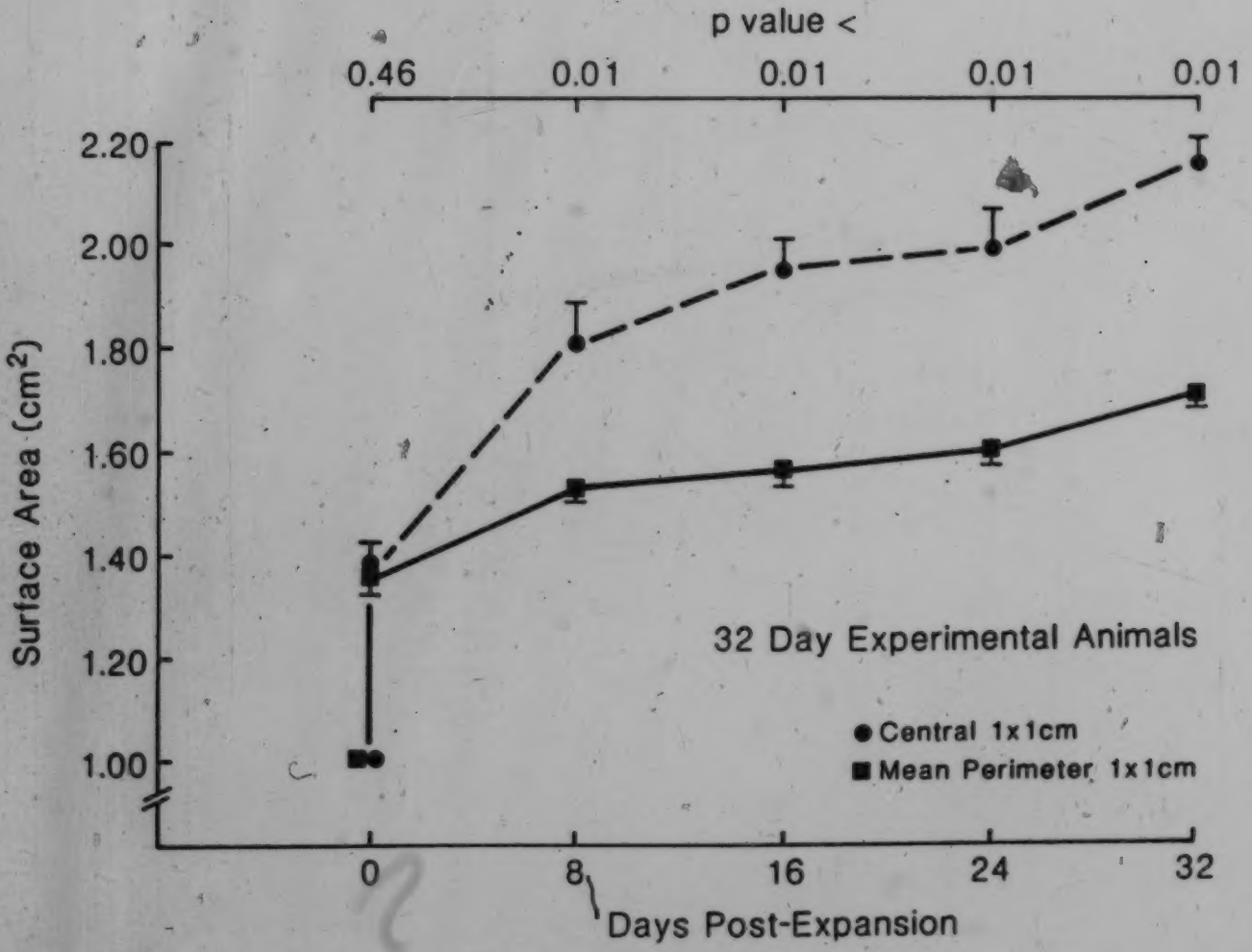
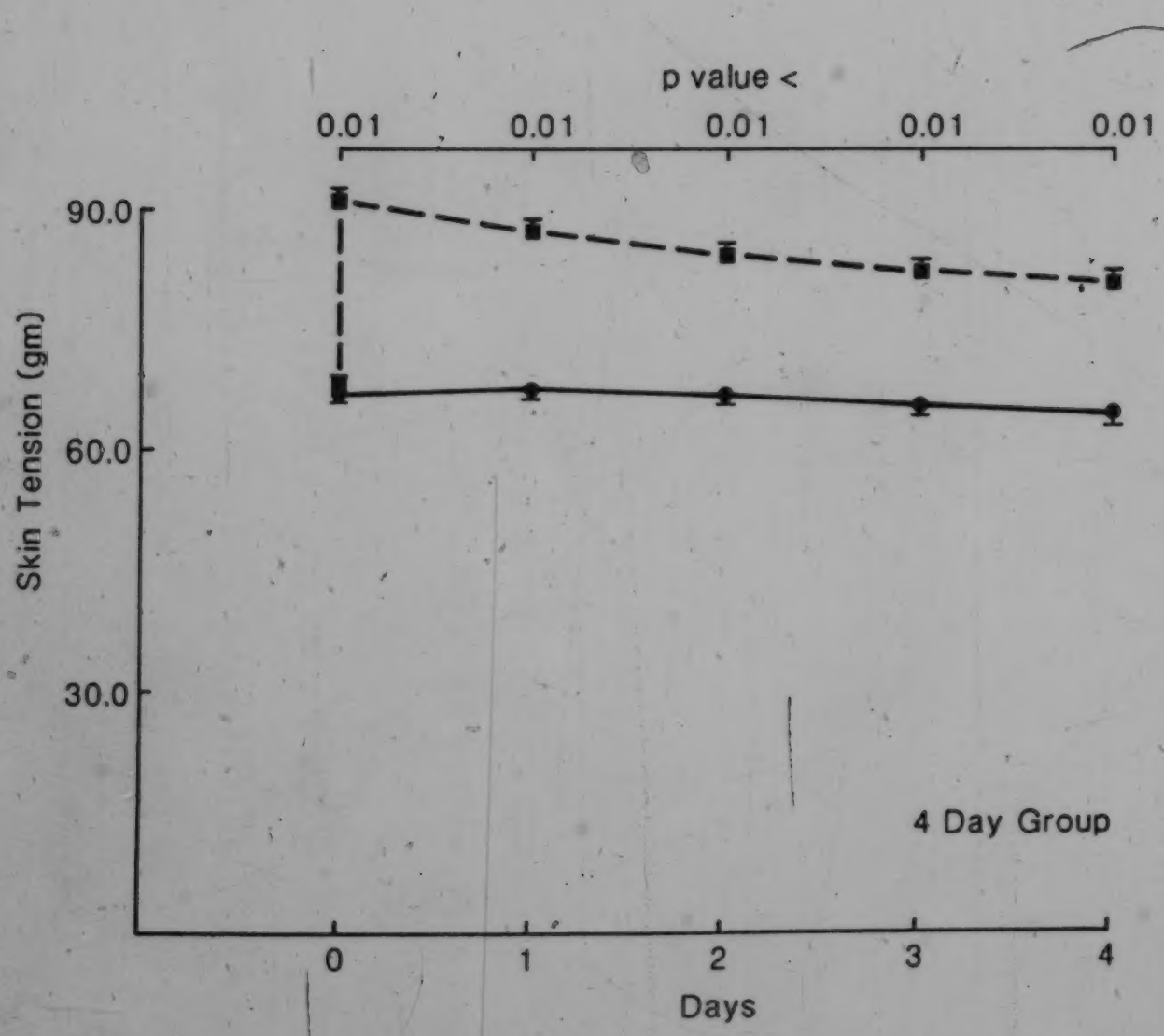

p value <
0.46
0.01
0.01
0.01
0.01
2.20
2.00
1.80
1.60
1.40
1.20
1.00
Surface Area (cm²)
32 Day Experimental Animals
Central 1x1cm
Mean Perimeter 1x1cm
0
8
16
24
32
Days Post-Expansion

FIGURE 15. A comparison of *in vivo* skin tension in experimental and control animals studied for 4 days in Project B. Standard errors of the means are represented by bars.

■ experimental animals

● control animals


p value <
0.01
0.01
0.01
0.01
0.01
90.0
60.0
30.0
Skin Tension (gm)
4 Day Group
0
1
2
3
4
Days

manner to 81.2 gm. The mean skin tension of the four day control animals decreased slightly in a curvilinear manner from 67.1 to 64.6 gm.

Mean skin tension in the eight day experimental animals rose from 68.4 to 90.5 gm immediately after inflation (FIGUkE 16) and then decreased in a curvilinear manner to 78.5 gm over eight days. Mean skin tension in the control 8 day animals decreased slightly from 66.9 to 63.9 gm. The means were not found to be significantly different.

In the sixteen day experimental animals the mean skin tensions rose initially to a value similar to those in the other groups and decreased to a value of 71.0 gm at day 16 (FIGURE 17). Mean skin tensions for the sixteen day control group decreased very slightly from 67.5 to 65.3 gm and this was not found to be statistically significant ($p<0.27$).

Skin tension of the 32 day experimental animals rose by approximately 36% to a value of 90.4 gm (FIGURE 18) and dcreased in an apparently linear manner to a value of 69.1 gm. Mean skin tension in the 32 day control animals decreased from 68.5 to 63.0 gm. The difference in mean skin tension between the experimental and control animals at 32 days was found to be significantly different.

FIGURE 16. A comparison of *in vivo* skin tension in experimental and control animals studied for 8 days in Project B. Standard errors of the means are represented by bars.

■ experimental animals

● control animals

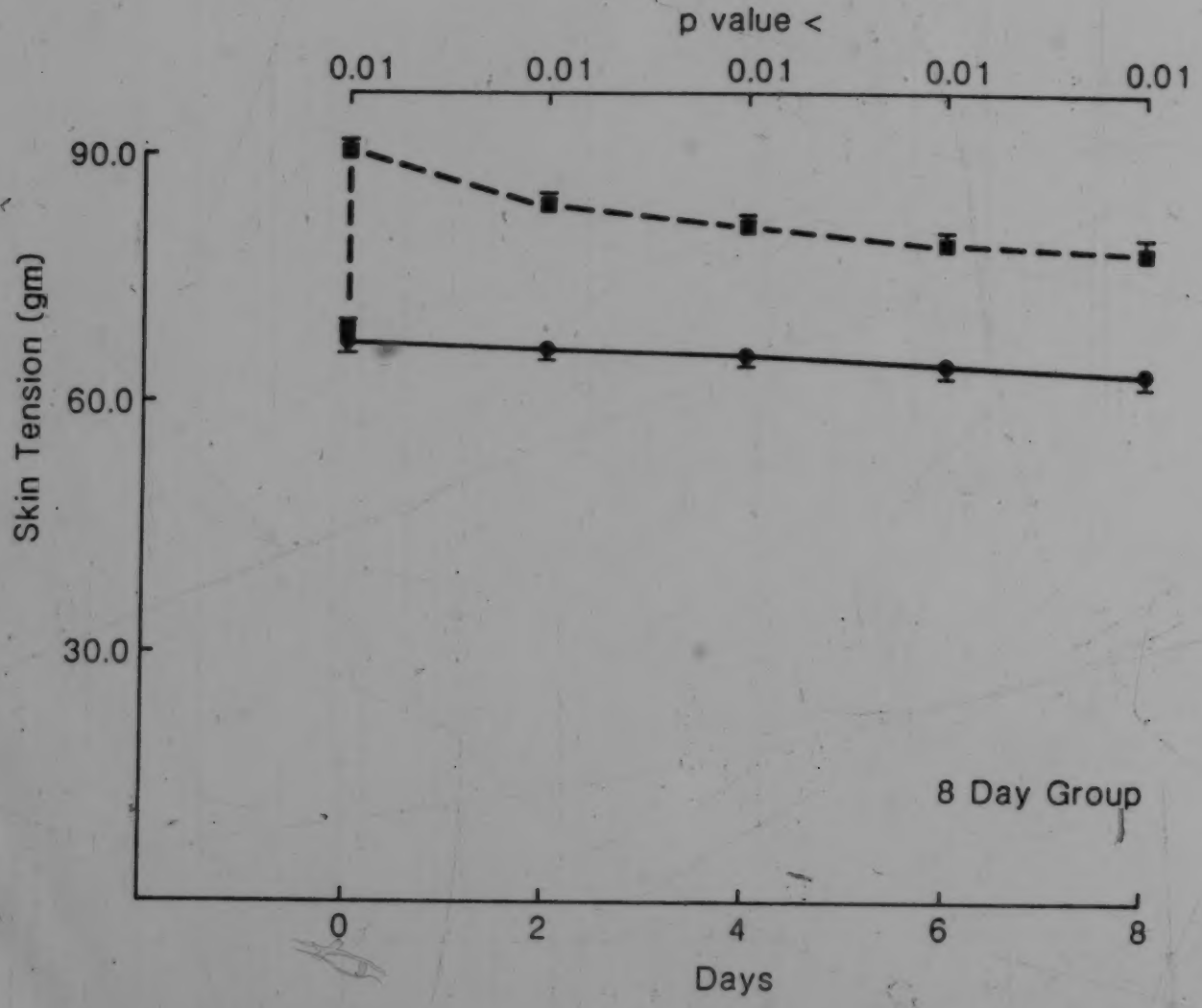

p value <
0.01
0.01
0.01
0.01
0.01
90.0
60.0
30.0
Skin Tension (gm)
8 Day Group
0
2
4
6
8
Days

FIGURE 17. A comparison of *in vivo* skin tension in experimental and control animals studied for 16 days in Project B. Standard errors of the means are represented by bars.

■ experimental animals

● control animals

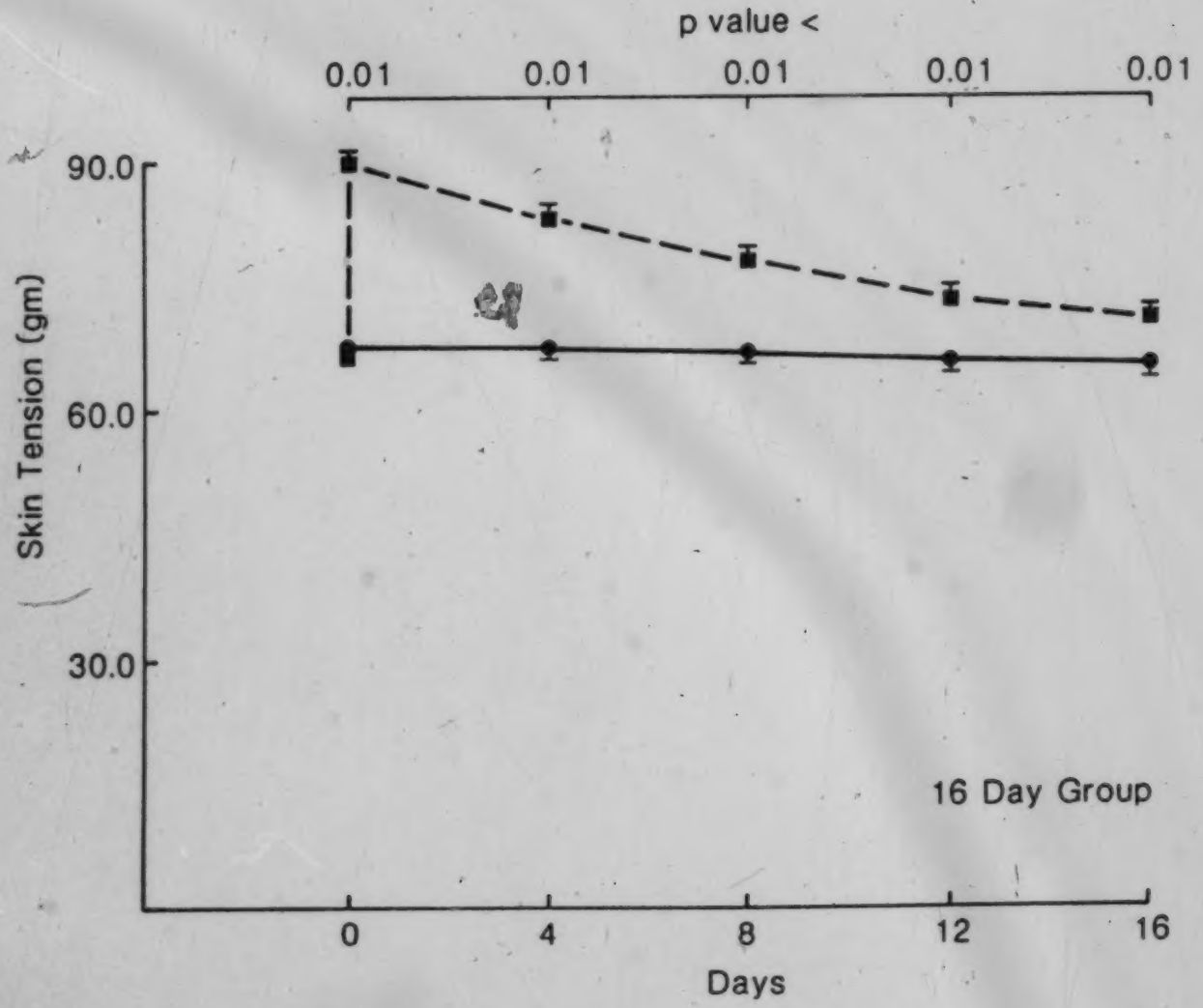
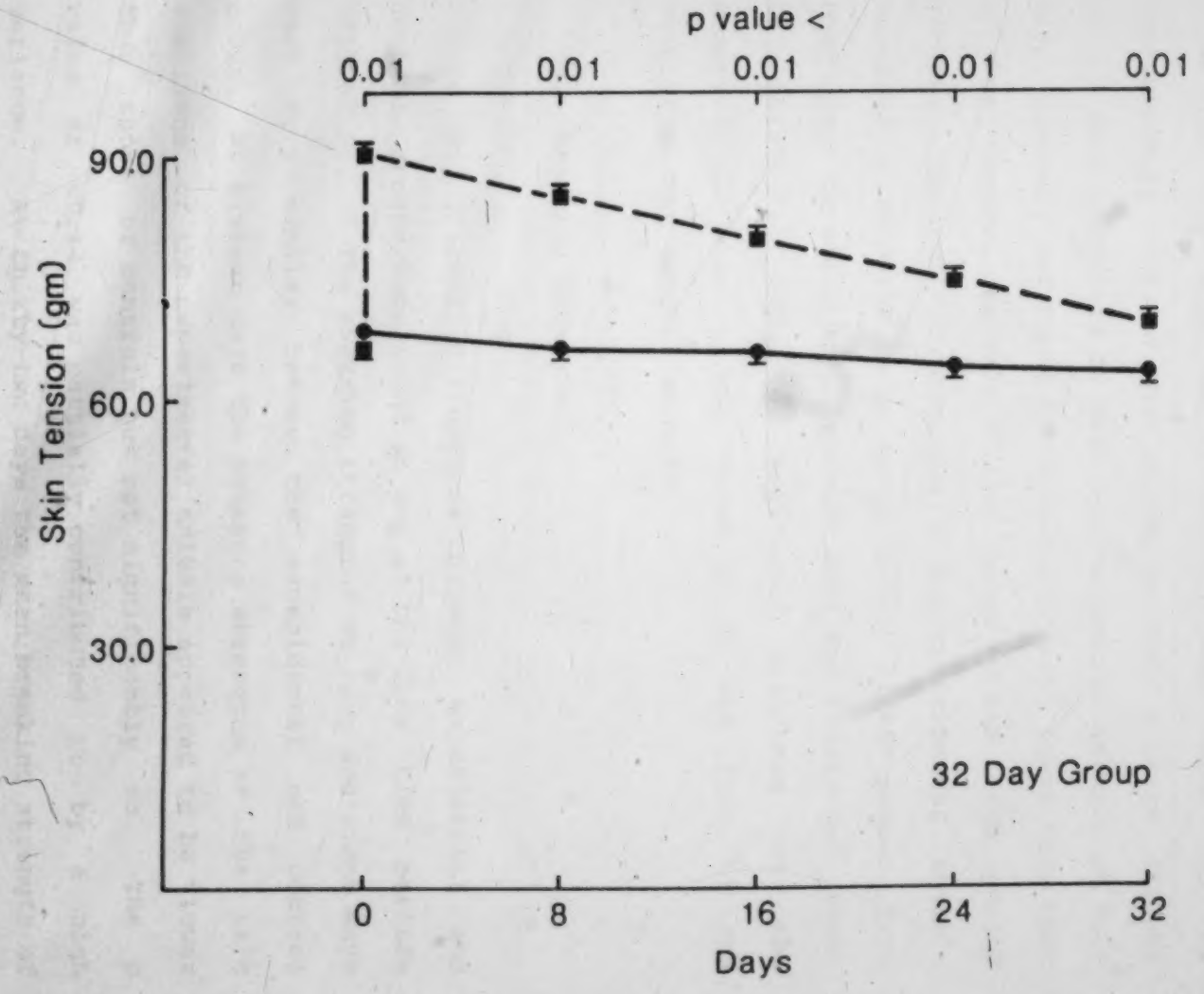

p value <
0.01
0.01
0.01
0.01
0.01
90.0
60.0
30.0
Skin Tension (gm)
16 Day Group
0
4
8
12
16
Days

FIGURE 18. A comparison of *in vivo* skin tension in experimental and control animals studied for 32 days in Project B. Standard errors of the means are represented by bars.

■ experimental animals

● control animals


p value <
0.01
0.01
0.01
0.01
0.01
90.0
60.0
30.0
Skin Tension (gm)
32 Day Group
0
8
16
24
32
Days

d. Skin Thickness

The mean thickness of skin at the time of sacrifice in the four day experimental animals was found to be approximately 78% of that in the control animals (FIGURE 19). Mean thickness of skin in the control animals at four days, however, was found to be significantly lower than that of the control animals of the three other time period groups. Mean skin thicknesses of the experimental animals appeared to increase in an approximately linear manner from four days to eight days, sixteen days and thirty-two days. At thirty-two days the mean skin thickness of the experimental animals was found to be 94% that of the thirty-two day control animals.

e. Breaking Strength

No significant differences between experimental and control groups were found at any of the four time periods (FIGURE 20). The breaking strengths at four and eight days were very similar between the experimental and control group. At sixteen days the breaking strengths of the skin specimens of the experimental animals appeared to be lower than those of controls but not significantly so. The p value of <0.14 was partially contributed to by a high variance. At thirty-two days the mean breaking strength of

FIGURE 19. A comparison of skin thicknesses of specimens from experimental and control animals in Project B. Standard errors of the means are represented by bars.

■ experimental animals

—— ● control animals

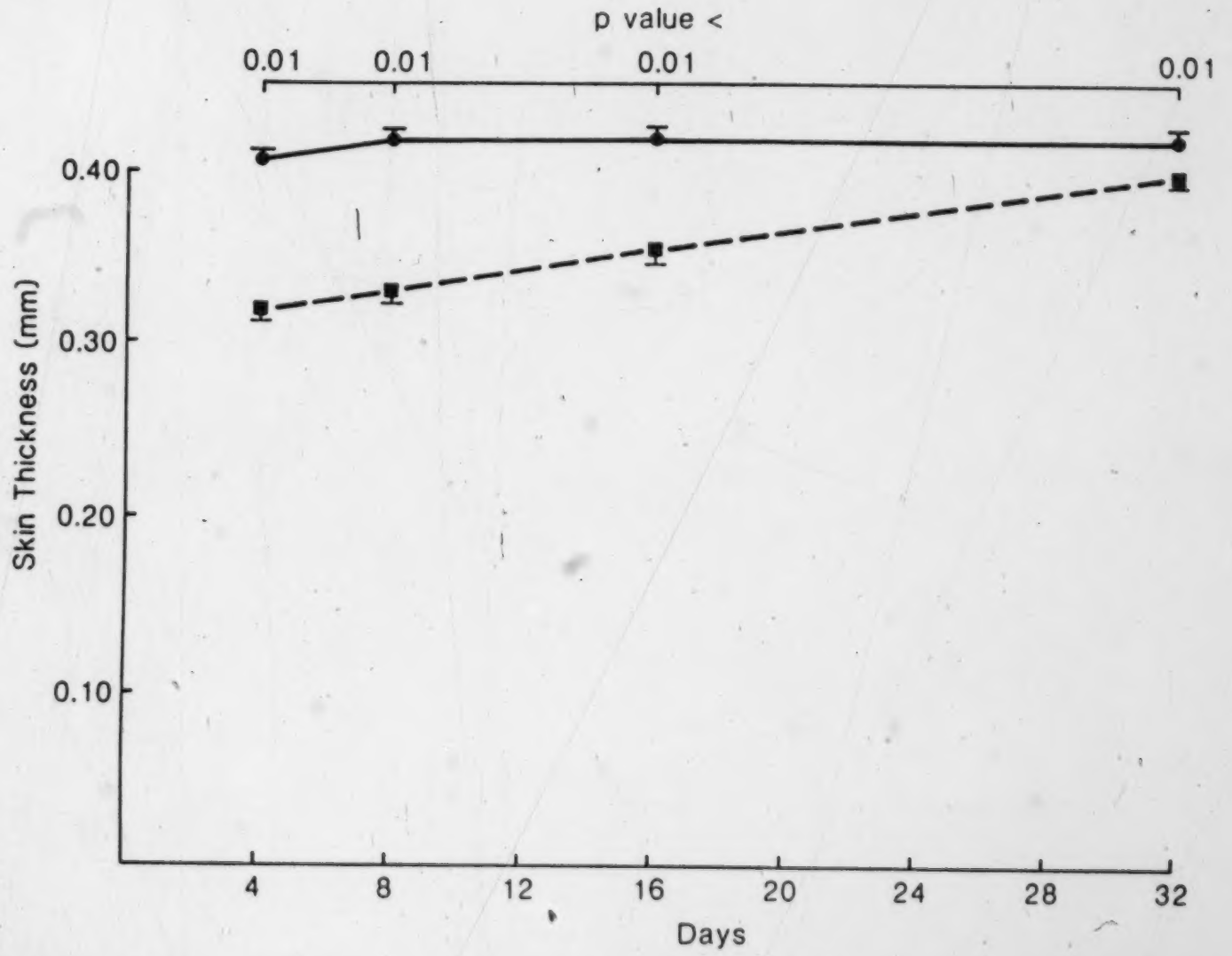
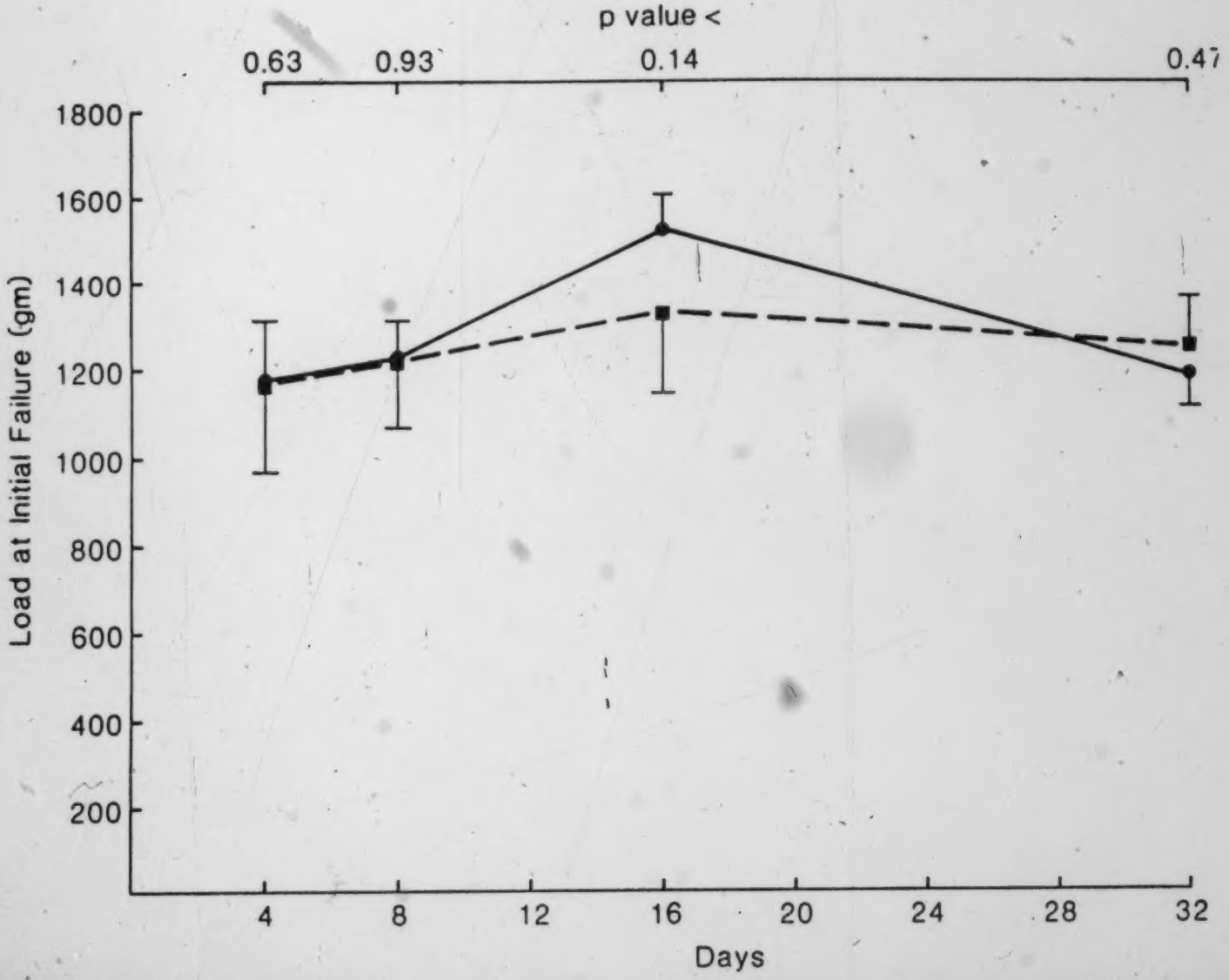

p value <
0.01
0.01
0.01
0.01
0.40
0.30
0.20
0.10
Skin Thickness (mm)
4
8
12
16
20
24
28
32
Days

FIGURE 20. A comparison of breaking strengths of skin specimens from experimental and control animals in Project B. Standard errors of the means are represented by bars.

■ experimental animals

● control animals


p value <
0.63
0.93
0.14
0.47
1800
1600
1400
1200
1000
800
600
400
200
Load at Initial Failure (gm)
4
8
12
16
20
24
28
32
Days

the specimens of the experimental animals was higher than that of the control animals, but was not found to be statistically significant.

f. Specimen Weights

Wet weights of the skin specimens of experimental animals were not found to be statistically different from those of control animals at four, eight, and sixteen days after expansion (FIGURE 21). At sixteen days it was noted that the mean wet weight of experimental animal skin specimens was 62% greater than that of the control animals. At thirty-two days after expansion the mean wet weight of skin specimens in the experimental group was found to be 118% greater than that of the control group.

Analysis of lyophilized (dry) weights revealed findings similar to those of wet weights (FIGURE 22). However, at four days the mean dry weight of skin specimens from experimental animals was found to be reduced 17% relative to controls. This was significantly different at a level of <0.04. No significant difference was noted in the mean dry weights at eight days after expansion. There was a significant difference between the experimental and control dry weights at 16 days. Mean dry weight of the experimental animals was found to be increased 86% over that of controls

FIGURE 21. A comparison of wet weights of skin specimens from experimental and control animals in Project B. Standard errors of the means are represented by bars.

■ experimental animals

● control animals

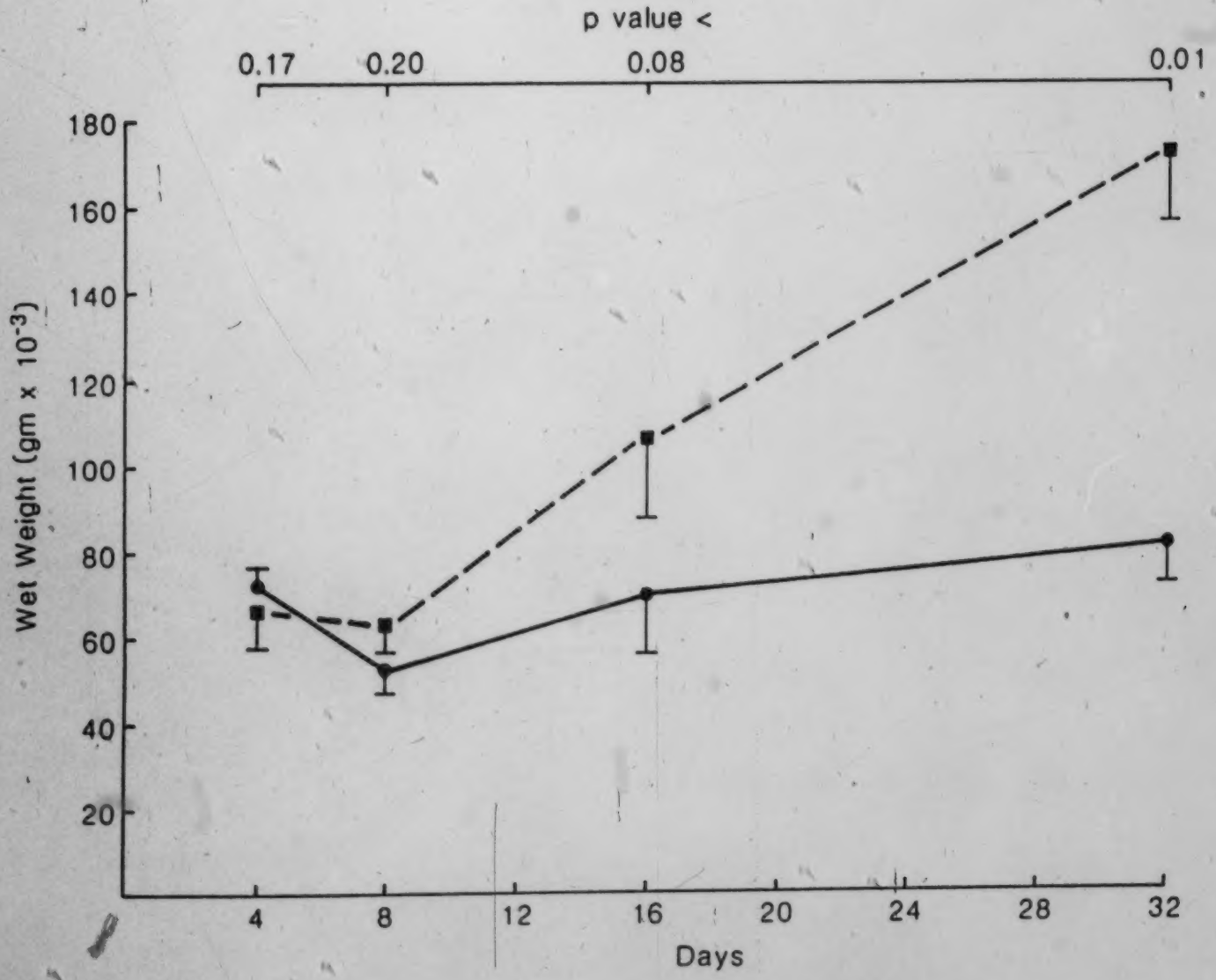
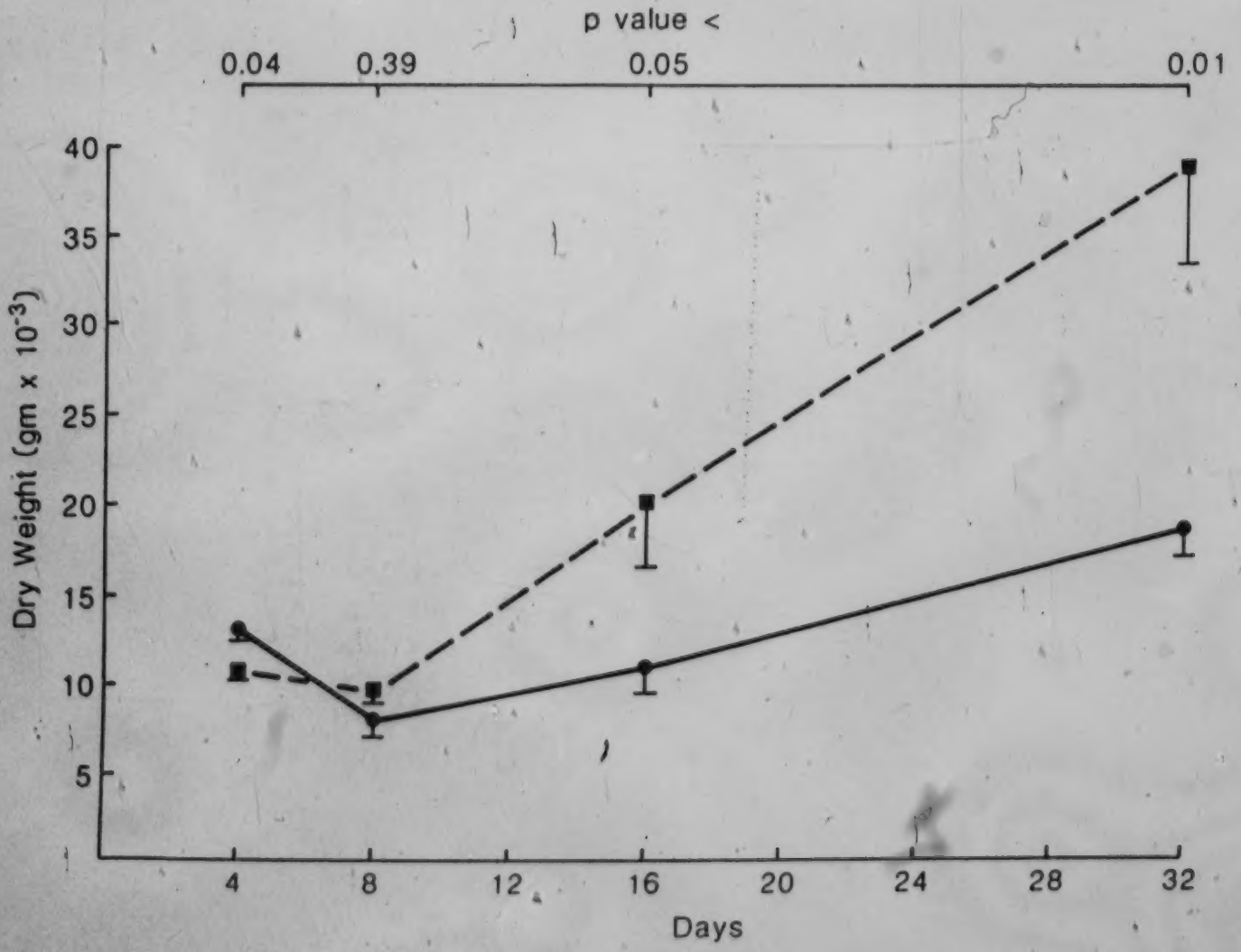

p value <
0.17
0.20
0.08
0.01
Wet Weight (gm x 10^{-3})
180
160
140
120
100
80
60
40
20
4
8
12
16
20
24
28
32
Days

FIGURE 22. A comparison of dry weights of skin specimens from experimental and control animals in Project B . Standard errors of the means are represented by bars.

■ experimental animals

● control animals


p value <
0.04
0.39
0.05
0.01
Dry Weight (gm x 10^{-3})
40
35
30
25
20
15
10
5
4
8
12
16
20
24
28
32
Days

at sixteen days.

Mean dry weight of skin specimens at thirty-two days after expansion was found to be 114% greater than that of control animals.

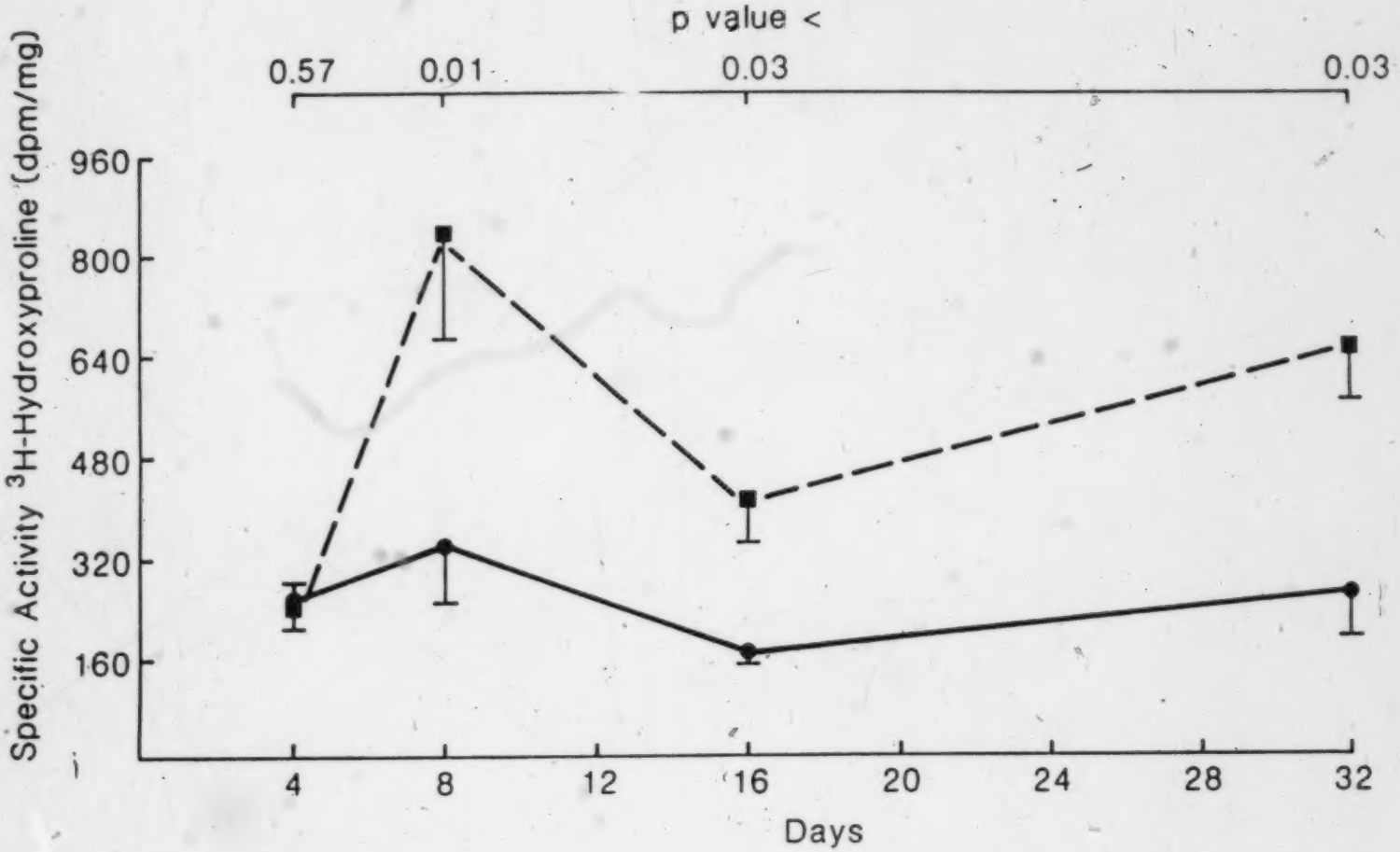
g. Specific Activity

There was no difference in specific activity of tritiated hydroxyproline in the skin specimens harvested from the experimental animals versus the control animals at four days after expansion (FIGURE 23). However, at eight days the specific activity in experimental animals was found to be significantly elevated relative to that of controls. Similarly, a significant difference in specific activity in the skin of experimental animals relative to controls was noted at sixteen days. Specific activity was also found to be elevated in the skin of experimental animals relative to control animals at 32 days. Of the three time periods at which specific activity was found to be elevated in the experimental animals, the highest specific activity was in the eight day group.

FIGURE 23. A comparison of ^{3}H hydroxyproline specific activities in skin specimens from experimental and control animals in Project B. Standard errors of the means are represented by bars.

■ experimental animals

● control animals


p value <
0.57
0.01
0.03
0.03
Specific Activity ^{3}H-Hydroxyproline (dpm/mg)
960
800
640
480
320
160
4
8
12
16
20
24
28
32
Days

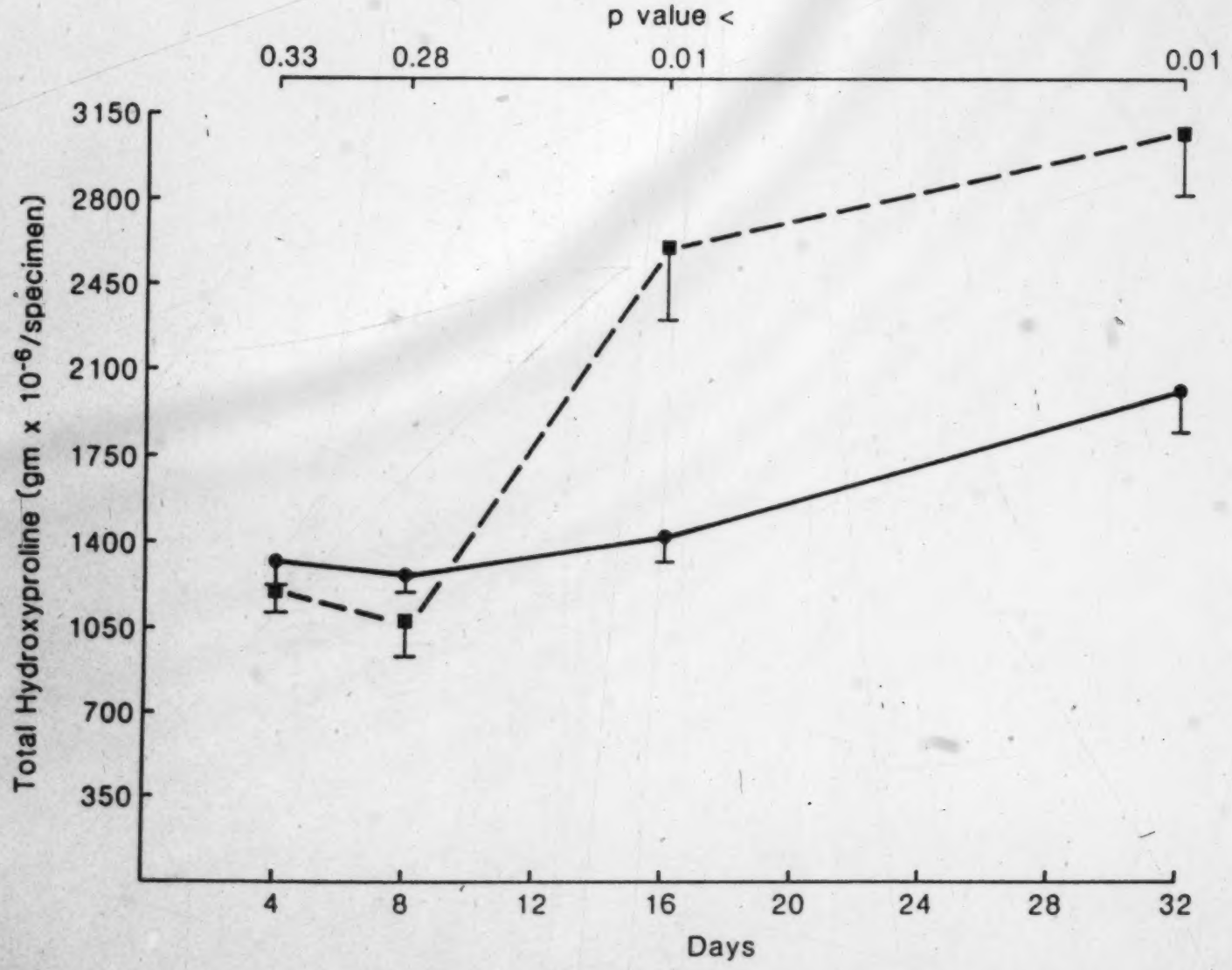
h. Total Hydroxyproline Content

An increase in total hydroxyproline in the skin of control animals was noted over 32 days (FIGURE 24). No significant difference was found in the total hydroxyproline content between experimental and control animals at four and eight days. The total hydroxyproline , however, was found to be significantly elevated in the 16 day and 32 day experimental groups, relative to controls. Total hydroxyproline content in the skin of animals at sixteen days after expansion was found to be elevated approximately 80% relative to that of control animals. Total hydroxyproline in the skin of the experimental animals at 32 days was found to be approximately 50% greater than that in control animals at thirty-two days. The rates of increase or accumulation of total hydroxyproline for the experimental and control groups between 16 and 32 days were similar.

FIGURE 24. A comparison of total hydroxyproline content of skin specimens from experimental and control animals in Project B. Standard errors of the means are represented by bars.

■ experimental animals

● control animals


p value <
0.33
0.28
0.01
0.01
3150
2800
2450
2100
1750
1400
1050
700
350
Total Hydroxyproline (gm x 10-6/specimen)
4
8
12
16
20
24
28
32
Days

IV DISCUSSION

A. Project A

The most important finding from Project A is that within eight days after induction of mechanical stress on the skin through therapeutic tissue expansion, collagen synthesis is maximally stimulated. At four days after expansion, there is an approximate six-fold increase in rate of collagen synthesis relative to normal unstressed skin. Collagen synthesis in stressed skin thereafter declines progressively such that by sixty-four days after expansion, it returns to normal.

The decline in collagen synthesis activity seen between four and eight days post expansion is probably due to decreased tensile stimulation. Relaxation is probably the result of either one or a combination of: visco-elastic creep[47], remodelling, or addition of new collagen. Expansion devices remained fully inflated throughout the experimental period.

Mechanical stress induced by therapeutic tissue expansion is probably in the form of compression of the dermis or tensile stress on the dermis, or both. Conceptually, the latter is the more likely to be

responsible for the increase in surface area, collagen synthetic activity and accumulation of total hydroxyproline content. Increase of *in vivo* skin tension during the process of therapeutic tissue expansion supports the idea that tensile stress is the primary stimulant of dermal changes during therapeutic expansion. However, compressive forces were not examined in this study because of a lack of a satisfactory technique to measure these *in vivo*.

Tensile stress has been demonstrated by other investigators to induce cellular changes. Hasegawa observed that when mechanical stretching was applied to cultured bone cells, either intermittently or continuously, over 2 hours, there was a 64% increase in the number of cells synthesizing DNA occurred[48]. Tensile stress applied to the bone cells also resulted in increased incorporation of tritiated proline and tritiated leucine. Brunette has demonstrated a rapid increase in the number of isolated epithelial cells synthesizing DNA when exposed to tensile stress *in vitro*[49]. Squier reported an increased mitotic index and progenitor cell population in epidermis of hairless mice stretched by implanted springs *in vivo*. He reported subsequent tissue hyperplasia to be maintained over 4 days after implantation[50]. Francis et al. observed a rise in thymidine autoradiographic labelling index in epidermis *in vivo* over silastic implants in guinea pigs[51]. Lorber et

al. studied effects of tension on rat epidermis *in vivo* by autoradiographic labelling and histologic examination[52]. They described histologic changes of cell elongation, flattening, separation and narrowing of subepithelial vessels. Slight stretching increased labelling but with further tension a point was reached beyond which labelling decreased. He ascribed this to relative ischemia. As previous, Yen et al. observed that when tensile stress was applied by an implanted spring to the cranial sutures of mice, a rise in hydroxyproline specific activity of the explanted sutures occurred[32]. Specific activity increased significantly 3 days after force application and remained higher than in controls for up to 14 days. Peak collagen synthetic activity was reported to occur at 3 days.

Blood urea nitrogens were studied to rule out metabolic derangement as a result of possible renal failure. This was the first time that an animal model for tissue expansion had utilized a device placed intra-abdominally. Although physiologically this is not unlike a pregnancy in a female rat, there was concern regarding compression of the urinary system or renal vessels, leading to possible obstructive renal failure. There was no evidence of this, however, neither at necropsy nor by blood urea nitrogen determination.

Examination of blood urea nitrogens of the control and experimental groups revealed no statistically significant differences at four, eight, sixteen or sixty-four days. There was a statistically significant elevation in experimental animals at thirty-two days but this is not considered to be clinically significant as both of the mean values of the control and experimental groups of 22.1 mg/dL and 19.0 mg/dL respectively are considered to be within the normal range of blood urea nitrogens for Sprague-Dawley rats[53].

Examination of breaking strengths of skin specimens in Project A suggests that for a given post-expansion specimen size, the skin undergoing therapeutic tissue expansion is initially compromised in its ability to withstand tensile loading. The reason for the apparent decreased values in load to initial failure for both the controls and experimental animals at sixteen days post-expansion is unclear. The possible causes for this apparent abberation may have been ill health of both of these groups of animals, a problem in preservation of the specimens (e.g. freezing) or perhaps miscalibration of the tensile testing device. However, there was no direct evidence of any one of these. High variance in each of the groups contributed to statistical insignificance between experimentals and controls at days sixteen, thirty-two and sixty-four. It is,

however, apparent from examination of FIGURE 1 that, at least initially, the ability of skin under the stress of therapeutic tissue expansion to withstand tensile load is compromised.

B. Project B

The differences in trends and weight patterns between the four time groups suggests that the animals were adversely affected by the frequency of the anaesthetic. There appeared to be a direct relationship between frequency of anesthetic and weight loss. This is especially noted in the animals which underwent daily anaesthesia for a period of four days. Both experimental and control animals of the 4 day group of Project B lost weight daily. Moreover, animals in the four day group of Project A demonstrated no such weight loss.

That frequent repetitive anaesthetics had an adverse effect on the animals is also supported by comparison of the collagen synthetic activities between the animals of Project A and Project B. - A dramatic increase in collagen synthetic activity was noted at day four post-expansion in Project A. No significant increase in synthetic activity was noted in the four day experimental animals of Project B subjected to anaesthetics. In Project B, an increase in collagen

synthetic activity was not noted until day eight after expansion. Examination of the body weights of these animals revealed that the 8 day animals which were subjected to the general anaesthetic every two days were able to maintain their body weight. It is also noted that collagen synthetic activity remained higher in experimental animals compared to control animals at thirty-two days in Project B. These findings suggest that frequent anaesthesia had the effect of shifting the curve of collagen synthetic activity in the skin of therapeutically expanded animals to the right, effectively delaying the response to tensile stress.

Frequent general anesthesia may have affected the metabolism of the animals of Project B in two ways. General anaesthetics , in particular, directly affect chemoreceptor trigger zones of the medulla oblongata inducing nausea and gastrointestinal upset[54]. Single dose exposures to general anesthetics in humans leads to an incidence of vomiting of 27 to 82 percent[55]. Despite its common use as a general anesthetic in small animal application, methoxyflurane has been shown to have a remarkably long whole body single dose wash-out time of 10 to 29 hours.[56] In the four day animals having received methoxyflurane every 24 hours this would have resulted in little central nervous system recovery over four days of repetitive exposure, with consequent anorexia and gastrointestinal upseet. A more

profound effet that repetitive methoxyflurane may have had on the metabolism of Project B animals is through free fluoride ion toxicity. Free fluoride is a product of the metabolism of methoxyflurane and can lead to renal failure. Fluoride toxicity has been shown to be a product of length and frequency of exposures which leads to a critical level above which toxicity occurs[57]. Its specific effect on wound metabolism does not appear to have been reported although free fluoride is metabolically toxic. Free fluoride inhibits many enzyme systems including those of the glycolytic pathway. Clinically this is characterized by weight loss and weakness[58.] Again the four day animals would be most affected in view of the slow wash-out time associated with methoxyflurane. It should be noted that no adverse effect on blood urea nitrogen by single dose exposure to methoxyflurane was observed in Project A.

It is conceivable that general anesthetic agent itself may have had a direct negative effect on collagen synthesis. Fauss et al.[59] reported that nitrous oxide and isoflurane inhibited ADP-induced platelet aggregation while O'Rourke et al.[60] and Evangelista et al.[61] demonstrated an inhibitory effect of barbiturates on platelet aggregation. Dalsgaard et al.[62] found that halothane impaired both ADP induced and collagen induced platelet aggregation. Because platelet aggregation is an important part of early wound

healing, it may be that inhibition of platelet aggregation indirectly adversely affected collagen synthesis - particularly in those animals subjected to the more frequent anesthetics. However, Kanta et al.[63] reported that topically administered local anesthetics increased hydroxyproline levels in wounded tissue. Vasseur et al.[64] reported no effect of lidocaine and bupivicaine on the breaking strength of wounds. Algie et al.[65] reported no effect on the general anesthetic agent nitrous oxide on wound healing.

Although it seems obvious that therapeutic expansion of skin should bring about an increase in surface area, this has been objectively demonstrated in this study. Examination of surface area changes reveals an initial approximately forty percent increase in skin surface area at the time of expansion. This is probably the direct result of biomechanical properties of the skin , particularly viscoelastic creep. However, a second phase of change in skin surface area with time has been demonstrated. Noted particularly in the sixteen day and thirty-two day group experimental animals, was a further increase in surface area. The gain in surface area realized immediately at the time of expansion was matched by an approximately equivalent increase in surface area over the remaining thirty-two days. The second phase of gain in surface area is probably the

result of either remodelling or the addition of connective tissue. The increase in surface area of approximately 11% over thirty-two days in the control animals probably reflects growth. This is also supported by the demonstrated weight gain of the thirty-two day animals. Both control and experimental animals of the four day groups appear to have undergone a slight decrease in surface area over four days. This may have been the result of a decrease in body weight experienced by both groups at four days.

Comparison of changes in surface areas of the central 1 x 1 cm. square compared to the mean perimeter 1 x 1 cm. square with time suggests that initially the two different areas of the skin overlying the expansion device contribute approximately equally to the gain in surface area, at the time of inflation. However, with time the second phase of increase of surface area is contributed to more by an increase in surface area of skin located centrally (over the apex of the expansion device), than those areas located more peripherally. This was particularly demonstrated in the thirty-two day group but was true for all four time groups. The finding of disproportionate contribution to increase in surface area agrees with Brobmann et al.[66] and a preliminary report by Leighton et al.[67] They also observed the greatest change in surface area to take place centrally during expansion in pigs. Neither report ,

however, commented on an apparent two phase increase in surface area demonstrated here. That the increase in surface area observed centrally was not the result of stretching of the wound was suggested by the lack of observation of widening of the incisional scar at the time of skin harvest. Furthermore, a similar differential increase in surface area was observed in skin areas located intermediately between the center area bearing the skin wound, and the perimeter - i.e. areas A and C (PLATE 6) (See APPENDIX C, Tables 14-17,22-29). There was a similar intermediate increase in surface area of intermediately located areas relative to the peripheral and central study areas.

Skin tension overlying the inflated expansion devices increased at the time of inflation, by approximately one-third of normal skin tension. With time, skin tension returned towards normal values.

It appears that the thickness of therapeutically expanded skin is compromised. This is particularly noted early during the expansion process. However, with time thickness tends to return towards normal.

Although the ability to withstand tensile stress was initially shown to have been compromised by tissue expansion in Project A , there were no statistical differences in the

breaking strengths of skin specimens between experimental and control groups in Project B. Project A examined a skin strip specimen 3 x 1 cm. in dimension after the process of skin expansion and the resultant increase in surface area had occurred. Project B examined a skin strip specimen which was 3 x 1 cm prior to therapeutic expansion and the resultant increase in surface area. It is interesting to note that for example in the four day group of Project B there was an approximately twenty-five percent decrease in thickness but an approximate thirty-three percent increase in surface area. If the skin specimen had increased in surface area proportionately in length and width, this would represent an approximate fifteen percent increase in width of the specimen. In tensile testing of materials, cross-sectional area of the specimen is an important factor, secondary only to the material properties of the specimen. Since in Project B it appears that for each time group, in the experimental specimens, a decrease in skin thickness was accompanied by an increase in width, these would be offsetting and would result in breaking strengths similar to those of control animals. This is indeed what was observed over the time periods. However, in view of information that we have that skin thickness increased towards normal with time and skin surface area increase persisted with time, as well as information that total hydroxyproline content increased with time, we eventually would expect to see an

increased mean breaking strength in the skin specimens in the experimental animals of Project B. This was not demonstrated although it is noted from FIGURE 20 that at thirty-two days, for the first time, mean breaking strengths of experimental animals were greater than those of control animals, although the increase was not statistically significant. It is possible that the new collagen accumulated is immature and poorly cross-linked. It may also have been because of a relative accumulation of Type V collagen as reported by Vander Kolk et al.[41]. However, the most likely explanation for the lack of an observation of increase in tensile strength in expanded tissue comes from Doillon et al.[69]. These workers studied wounded guinea pig skin under tensile stress and reported that during the first 28 days of collagen synthesis, although increased numbers of collagen fibers were observed by scanning electron microscopy, these were of much smaller diameter than normal. Between 28 and 90 days after wounding, few new collagen fibers appeared but individual collagen fibers increased in diameter and this correlated with the remodelling phase of wound healing. At 180 days the diameter of the collagen fibers and fiber morphology was observed to be normal. There was a positive direct relationship between collagen fiber diameter and wound tensile strength.

Specific activity of tritiated hydroxyproline is presently considered to be the most satisfactory method to study collagen synthetic activity. In view of the results of Project A the fact that specific activity in the skin of experimental animals at thirty-two days was still elevated relative to control animals was initially unexpected. However, it appears that in these animals at thirty-two days, there was still a stimulus to synthesize collagen, in that mean skin thickness was approximately five percent less than normal, and skin tension remained elevated approximately 9% above normal.

The observation of a modest increase in total hydroxyproline content in the specimens of control animals concurs with Fry et al.[70]. They reported an increase in tensile strength in the skin of rats with age, in part due to an increase in collagen content with age.

The demonstration of increased wet weights and dry weights of skin samples with time in the experimental animals supports the concept that there is accumulation of new tisssue. The tissue is collagenous as evidenced by increases in hydroxyproline content. Collagen degradation in therapeutically expanded tissue has not been studied. However, collagen synthesis and a net accumulation of collagen in response to therapeutic dermal expansion have

been demonstrated.

With this information regarding differential and biphasic contributions to surface area, increase in collagen synthesis and net increase in collagen (new tissue in response to tissue expansion), as well as resultant decrease in skin tension and a return of skin thickness toward normal, the reconstructive surgeon may more accurately plan soft tissue reconstruction with more predictable clinical results. This knowledge should not only lead to more predictable results in the quality of the final reconstruction but also decrease the frequency and severity of complications encountered toward that end.

If new tissue is generated in response to tissue expansion in the form of collagen, what then is the physiologic and clinical limit? The answer yet remains unclear. Results of the research reported herein suggest in the long term the limit may not be thinning or lack of tissue. While basic research continues, larger clinical soft tissue defects are being reconstructed by means of tissue expansion[68]. A part of the future of reconstructive surgery lies in the complete basic science answer to the question of the limit of tissue expansion.

V CONCLUSION

A model for the study of the effects of therapeutic tissue expansion on the skin of rats has been developed. The model used a Dow Corning self-contained-valve tissue expansion device inserted into the peritoneal cavity of Sprague-Dawley rats. No adverse effects were noted at least as evidenced by body weights and blood urea nitrogens.

In the dermis of skin subjected to therapeutic tissue expansion the following changes occur. Immediately there is an increase in tension and surface area overlying the expansion device. Skin tension decreases with time within 24 hours after expansion.

The increase in surface area occurs in two phases. The first is an immediate increase brought about at the time of expander inflation. A second slower phase occurs thereafter. Initially a large area of skin participates in the surface area change but with time skin located most centrally over the apex of the expansion device contributes maximally to the increase in surface area.

Although initially compromised , the thickness of skin subjected to therapeutic tissue expansion returns towards normal.

Collagen synthesis is stimulated by therapeutic tissue expansion. With time there is a net increase in collagen as evidenced by increased total hydroxyproline. All of the changes noted are probably the result of cellular and physiologic mechanisms which attempt to return toward normal the local cellular environment with respect to pressure and or tension. The responses observed are probably homeostatic mechanisms directed towards maintenance of normal pre-stress pressure , skin tension or skin mass per unit area.

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APPENDIX A

Three Dimensional Digitizer

APPENDIX A

Three Dimensional Digitizer[1]

Overview

This document is intended to give a brief description of the design, development and application of a micro processor controlled three dimensional surface scanner.

The device itself has been developed by Professor A.E. Peterson of the Civil Engineering Department and is based on an earlier scanner built to contour scour holes in hydraulic studies. The river scanner was much modified and refined for precision use on small animals.

System Requirements

1. The scanner had to be able to generate an accurate surface area model of the subject's (in this case the subjects would be white rats) abdominal area. The abdominal area to be scanned would be a square approximately 5 centimeters on a side. The surface would be continuous and more or less uniform in texture and coloration.

2. In addition, the scanner had to be able to locate specific reference points on the test animal and in

[1] written in conjunction with Robert McLaren, undergraduate student, Faculty of Engineering, University of Alberta

processing the data certain surface areas had to be calculated using the reference points as boundaries.

3. The scanner had to gather its data in a manner that would not disturb or deform the rat's delicate abdominal area. i.e. indirect sensing was required.

4. The complete scanning process had to be completed in as short a time as possible, since the test subjects had to be kept under a general anesthetic while undergoing the scan. Due to the nature of the anesthetic, it was in the interest of the animals to keep them under for as short a time as possible.

System Design

With the requirements in mind and the fact that an operational scanner was available, we went about the process of transforming the basic three dimensional scanner into an integrated system that would meet all of the specifications.

Hardware Configuration

The actual physical scanner is modeled on a three axis (X,Y,Z) orthogonal coordinate system.

X,Y Axis Control

The X and Y axes are implemented using a matched

pair of UniSlide Motor Driven Assemblies. Each assembly consists of a step motor driven worm gear running the length of it which is used to move the mounting bracket. In this particular configuration, the assembly that provides the X axis motion is mounted directly to the slider of the bed which provides the Y axis motion. In this manner by varying the location of the X and Y axis slides, any (X,Y) coordinate can be reached. The range of the two arm system is 11.8 inches in the Y direction and 9.5 inches in the X direction with a resolution of 0.005 inch.

Z Axis Control

Mounted to the slider of the X axis arm is the optical feedback probe used to gather the Z axis data.

The probe consists of a tube containing a fiber optic cable. The tube is connected via a chain and sprocket drive to a servo motor which can adjust the elevation of the probe. At the top of the probe, part of the fiber optic bundle has been separated from the main body of the cable. A small light is connected to the top of the tube so that it shines down the main body of the fiber and a photo diode is also mounted at the top of the tube so that it is triggered by any light returning up the separated fibers. The output from the diode is fed into a balancing circuit. The other input to the balancing circuit is a reference level voltage. The output of the balance circuit is connected to the servo

motor controlling the height of probe.

Simply speaking, the balancing circuit works by adjusting the probe height (by varying the output to the servo motor) until the signal being received from the photo diode is equal to the reference signal. For example, if the probe is too close to the surface, the light intensity being reflected up the tube is more intense. This, in turn, causes the output from the photo diode to exceed the reference signal level and the circuit turns the servo motor on, raising the probe until the two input signals balance.

The probe height is measured using a rotary potentiometer connected to one of the sprockets. As the probe moves up and down the output of a reference signal fed into the potentiometer varies with the number of rotations of the sprocket. The output voltage was found to vary linearly with the probe height.

Scanner Control

The complete scanner was interfaced to an IBM XT micro computer. The computer provided two functions. First, it controlled the motion of the scanner and second, it was used to collect and record the XYZ coordinates.

The motion of the scanner was controlled by the software which sent trigger pulses to the stepper motors via an RS-232C serial interface. A joystick was also integrated into the system to allow for manual positioning of the

probe. By counting the number of step pulses sent to the step motors the software always knows the X and Y coordinates of the probe.

The output from the rotary potentiometer on the fiber optic probe was fed into an Analog to Digital Converter and in this way the Z coordinate could be calculated.

Testing and Calibration

Once the construction and integration of all of the hardware components was complete, it was necessary to verify the performance of the system.

Step Motor Testing

The step motors were tested by driving the XY arms over a series of calibrated courses and measuring the error at the completion of the course. In this manner the error in the XY coordinates was found to be so small that it did not require any correction.

Optical Feedback Probe Testing

The optical probe was put through a series of tests to measure its performance. Several aspects of its operation were investigated.

1. Color and texture sensitivity - Since the probe height above the object was dependent upon the amount of light reflected back up the fiber optic tube, it was found

that surface color and texture affected the actual distance between the probe and the object. If the object was of uniform color and texture, the performance of the probe was found to be acceptable.

The probe therefore was capable of providing accurate information about the relative elevation differences on an object but not its absolute elevation with respect to some remote datum.

2. Slope of surface - Again it was determined that as the slope of the surface increased the reflected light received by the probe decreased. This resulted in the absolute distance between the probe and the object varying as the slope of the surface.

After measuring the results on many surfaces a polynomial correction factor was found that provided a good recreation of the original object.

3. Discontinuities - It was found that because of the manner in which the probe operated, it could not reproduce discontinuities in a surface. The only solution found was to avoid scanning such a surface. This was not a problem for our application, however, since the rat's abdomen provided a smooth, continuous surface.

Software Development and Data Analysis

Having a working scanner with known operating parameters and limitations, an operating system could be designed and implemented to control the operation of the probe and the subsequent analysis of the data.

Operating System

A software package was developed that allowed the operator of the scanner to calibrate the probe, calibrate the joystick and customize the parameters of the scan itself. This included setting the area of the scan and the resolution. The raw scan data which consisted of X,Y,Z triplets along with optional comments was then written to a data file on a disk.

Post Processing and Analysis of Data

The next part of the software package was a program that applied the slope correction to the data. At this point the data was transferred to the Amdahl main frame computer for the numerical and statistical analysis. The final piece of software processed all of the data files gathered during the course of the study and calculated the desired surface areas, the changes in the specified areas, and totals for specific groups of data. It also performed some basic statistical calculations on the data.

APPENDIX B

Data from Project A

APPENDIX B

Data from Project A

Table 4

Breaking Strengths
(gm, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,c]	p value[d] (<)
4	1224±48	1715±195	12	0.02
8	1586±97	2000±216	13	0.03
16	904±89	1150±117	12	0.20
32	1315±389	2420±198	10	0.09
64	1665±177	2340±335	11	0.14

Table 5

Hydroxyproline Specific Activity
(^{3}H d.p.m./mg dry weight, mean ± 1s.e.m.)

Days	Experimental	Control	n[b,c]	p value[d] (<)
4	1988±650	336±24	12	0.01
8	696±92	169±2	13	0.01
16	534±69	205±21	12	0.02
32	518±76	281±46	10	0.07
64	197±35	337±42	11	0.14

b) total of experimentals and controls in each time-group

c) a total of 12 animals were lost due to anaesthetic(7) and post-operative(5) deaths

d) by Mann-Whitney test

APPENDIX C

Data from Project B

APPENDIX C

Data from Project B

Table 6

Body Weights of 4 Day Group
(gm, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b]	p value[d] (<)
0	275±8	267±4	12	0.12
1	272±8	265±4	12	0.15
2	264±7	260±4	12	0.46
3	257±6	253±4	12	0.63
4	249±6	249±5	12	0.85

Table 7

Body Weights of 8 Day Group
(gm, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,e]	p value[d] (<)
0	279±9	280±4	10	0.84
2	279±9	280±4	10	0.65
4	280±7	280±4	10	0.76
6	280±6	280±4	10	0.48
8	279±5	281±4	10	0.38

b) total of experimentals and controls in each time-group

c) a total of 12 animals were lost due to anaesthetic(7) and post-operative(5) deaths

e) 2 animals were lost due to anaesthetic and deaths

APPENDIX C

Data from Project B

Table 8

Body Weight of 16 Day Group
(gm, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,f]	p value[d] (<)
0	278±10	276±6	6	0.86
4	281±9	281±6	6	0.94
8	284±9	284±7	6	0.91
12	285±8	289±6	6	0.44
16	286±9	291±5	6	0.29

Table 9

Body Weight of 32 Day Group
(gm, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,g]	p value[d] (<)
0	267±5	265±5	10	0.59
8	271±4	271±4	10	0.94
16	274±4	277±5	10	0.28
24	277±4	284±4	10	0.08
32	280±4	290±5	10	0.06

b) total of experimentals and controls in each time-group

f) 6 animals were lost due to anesthetic(4) and post-operative(2) deaths

g) 2 animals were lost due to anaesthetic deaths

APPENDIX C

Data from Project B

Table 10

Skin Tension of 4 Day Group
(gm, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b]	p value[d] (<)
Pre-expansion	67.4±0.7	67.0±0.6	12	0.01
0	91.1±0.5	-	-	-
1	88.3±0.9	67.4±0.7	12	0.01
2	84.6±0.7	66.7±0.4	12	0.01
3	85.6±1.0	65.4±0.4	12	0.01
4	81.2±0.8	64.6±0.4	12	0.01

Table 11

Skin Tension of 8 Day Group
(gm, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,e]	p value[d] (<)
Pre-expansion	68.3±0.6	66.9±0.5	10	0.01
0	90.5±0.9	-	-	-
2	84.2±0.5	66.5±0.4	10	0.01
4	81.2±0.7	65.6±0.6	10	0.01
6	79.6±0.8	64.3±0.6	10	0.01
8	78.5±0.7	63.9±0.9	10	0.01

b) total of experimentals and controls in each time-group

d) by Mann-Whitney test

e) 2 animals were lost due to anaesthetic and deaths

APPENDIX C

Data from Project B

Table 12

Skin Tension of 16 Day Group
(gm, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,f]	p value[d] (<)
Pre-expansion	67.1±0.9	67.6±0.9	6	0.01
0	90.2±1.0	-	-	-
4	85.6±0.8	67.4±0.6	6	0.01
8	78.3±1.2	66.6±0.7	6	0.01
12	73.0±1.6	66.0±0.5	6	0.01
16	71.1±1.3	65.3±0.4	6	0.01

Table 13

Skin Tension of 32 Day Group
(gm, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,g]	p value[d] (<)
Pre-expansion	66.4±0.7	68.5±0.9	10	0.01
0	90.4±0.8	-	-	-
8	85.4±0.4	66.2±0.8	10	0.01
16	80.0±0.8	65.7±0.9	10	0.01
24	74.8±0.5	63.8±0.5	10	0.01
32	69.1±0.6	63.0±0.7	10	0.01

d) by Mann-Whitney test

f) 6 animals were lost due to anesthetic (4) and post-operative (2) deaths

g) 2 animals were lost due to anaesthetic deaths

APPENDIX C

Data from Project B

Table 14

Surface Area Lateral 3 x 1 cm Strip 4 Day Group
(relative to pre-expansion area, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b]	p value[d] (<)
Pre-expansion	1.00±0	-	6	-
0	1.40±0.06	1.00±0	12	0.01
1	1.41±0.05	1.17±0.05	12	0.01
2	1.41±0.06	1.13±0.03	12	0.01
3	1.46±0.07	1.05±0.04	12	0.01
4	1.42±0.08	1.03±0.02	12	0.01

Table 15

Surface Area Lateral 3 x 1 cm Strip 8 Day Group
(relative to pre-expansion area, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,e]	p value[d] (<)
Pre-expansion	1.00±0	-	4	-
0	1.41±0.07	1.00±0	10	0.01
2	1.44±0.05	1.05±0.06	10	0.01
4	1.46±0.05	1.08±0.07	10	0.01
6	1.52±0.05	1.17±0.07	10	0.02
8	1.48±0.07	1.07±0.06	10	0.01

b) total of experimentals and controls in each time-group

d) by Mann-Whitney test

e) 2 animals were lost due to anaesthetic and deaths

APPENDIX C

Data from Project B

Table 16

Surface Area Lateral 3 x 1 cm Strip 16 Day Group
(relative to pre-expansion area, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b]	p value[d] (<)
Pre-expansion	1.00±0	-	3	-
0	1.30±0.09	1.00±0	6	0.01
4	1.35±0.11	1.11±0.02	6	0.04
8	1.32±0.12	1.24±0.04	6	0.54
12	1.42±0.09	1.25±0.05	6	0.18
16	1.43±0.13	1.22±0.05	9	0.22

Table 17

Surface Area Lateral 3 x 1 cm Strip 32 Day Group
(relative to pre-expansion area, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,g]	p value[d] (<)
Pre-expansion	1.00±0	-	5	-
0	1.51±0.04	1.00±0	10	0.01
8	1.71±0.08	1.21±0.01	10	0.01
16	1.75±0.06	1.12±0.06	10	0.01
24	1.75±0.09	1.15±0.07	10	0.01
32	1.91±0.10	1.16±0.11	10	0.01

b) total of experimentals and controls in each time-group

d) by Mann-Whitney test

g) 2 animals were lost due to anaesthetic and deaths

APPENDIX C

Data from Project B

Table 18

Surface Area 5 x 5 cm Grid 4 Day Group
(relative to pre-expansion area, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b]	p value[d] (<)
Pre-expansion	1.00±0	-	6	-
0	1.33±0.02	1.00±0	12	0.01
1	1.33±0.03	1.07±0.02	12	0.01
2	1.33±0.05	1.06±0.02	12	0.01
3	1.33±0.05	0.98±0.02	12	0.01
4	1.31±0.04	0.96±0.03	12	0.01

Table 19

Surface Area 5 x 5 cm Grid 8 Day Group
(relative to pre-expansion area, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,e]	p value[d] (<)
Pre-expansion	1.00±0	-	4	-
0	1.36±0.03	1.00±0	10	0.01
2	1.37±0.05	1.03±0.06	10	0.01
4	1.39±0.05	1.03±0.05	10	0.01
6	1.43±0.05	1.10±0.07	10	0.02
8	1.42±0.06	1.10±0.06	10	0.01

b) total of experimentals and controls in each time-group

d) by Mann-Whitney test

e) 2 animals were lost due to anaesthetic and deaths

APPENDIX C

Data from Project B

Table 20

Surface Area 5 x 5 cm Grid 16 Day Group
(relative to pre-expansion area, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,e]	p value[d] (<)
Pre-expansion	1.00±0	-	3	-
0	1.30±0.04	1.00±0	6	0.01
4	1.28±0.06	1.03±0.02	6	0.01
8	1.30±0.08	1.15±0.05	6	0.18
12	1.41±0.07	1.15±0.04	6	0.03
16	1.43±0.10	1.16±0.05	6	0.05

Table 21

Surface Area 5 x 5 cm Grid 32 Day Group
(relative to pre-expansion area, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,e]	p value[d] (<)
Pre-expansion	1.00±	-	5	-
0	1.40±0.03	1.00±0	10	0.01
8	1.60±0.05	1.16±0.04	10	0.01
16	1.69±0.07	1.14±0.06	10	0.01
24	1.72±0.07	1.18±0.06	10	0.01
32	1.83±0.05	1.19±0.09	10	0.01

b) total of experimentals and controls in each time-group

d) by Mann-Whitney test

e) 2 animals were lost due to anaesthetic and deaths

APPENDIX C

Data from Project B

Table 22

Surface Area Central 1 x 1 cm 4 Day Group
(relative to pre-expansion area, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,e]	p value[d] (<)
Pre-expansion	1.00±0	-	6	-
0	1.31±0.02	1.00±0	12	0.01
1	1.30±0.01	1.12±0.01	12	0.01
2	1.38±0.04	1.07±0.01	12	0.01
3	1.38±0.03	1.03±0.01	12	0.01
4	1.43±0.03	1.06±0.01	12	0.01

Table 23

Surface Area Central 1 x 1 cm 8 Day Group
(relative to pre-expansion area, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,e]	p value[d] (<)
Pre-expansion	1.00±0	-	4	-
0	1.24±0.03	1.00±0	10	0.01
2	1.36±0.04	0.96±0.02	10	0.01
4	1.49±0.01	1.00±0.02	10	0.01
6	1.56±0.04	1.15±0.02	10	0.01
8	1.62±0.04	1.19±0.03	10	0.01

b) total of experimentals and controls in each time-group

d) by Mann-Whitney test

e) 2 animals were lost due to anaesthetic and deaths

APPENDIX C

Data from Project B

Table 24

Surface Area Central 1 x 1 cm 16 Day Group
(relative to pre-expansion area, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,f]	p value[d](<)
Pre-expansion	1.00±0	-	3	-
0	1.36±0.04	1.00±0	6	0.01
4	1.46±0.03	1.04±0.05	6	0.01
8	1.52±0.06	1.11±0.05	6	0.01
12	1.63±0.05	1.18±0.05	6	0.01
16	1.65±0.08	1.20±0.04	6	0.01

Table 25

Surface Area Central 1 x 1 cm 32 Day Group
(relative to pre-expansion area, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,g]	p value[d](<)
Pre-expansion	1.00±0	-	5	-
0	1.37±0.05	1.00±0	10	0.01
8	1.81±0.08	1.19±0.04	10	0.01
16	1.95±0.07	1.12±0.04	10	0.01
24	1.99±0.09	1.19±0.05	10	0.01
32	2.16±0.06	1.13±0.04	10	0.01

b) total of experimentals and controls in each time-group

f) 6 animals were lost due to anesthetic(4) and post-operative(2) deaths

g) 2 animals were lost due to anaesthetic deaths

APPENDIX C

Data from Project B

Table 26

Surface Area 1 cm Perimeter of Grid 4 Day Group
(relative to pre-expansion area, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b]	p value[d] (<)
Pre-expansion	1.00±0	-	6	-
0	1.32±0.01	1.00±0	12	0.01
1	1.31±0.01	1.05±0.01	12	0.01
2	1.31±0.02	1.04±0.01	12	0.01
3	1.28±0.02	0.96±0.01	12	0.01
4	1.27±0.02	0.93±0.01	12	0.01

Table 27

Surface Area 1 cm Perimeter of Grid 8 Day Group
(relative to pre-expansion area, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,e]	p value[d] (<)
Pre-expansion	1.00±0	-	4	-
0	1.36±0.01	1.00±0	10	0.01
2	1.35±0.03	1.02±0.02	10	0.01
4	1.34±0.02	1.01±0.02	10	0.01
6	1.38±0.03	1.08±0.03	10	0.01
8	1.32±0.03	1.08±0.03	10	0.01

b) total of experimentals and controls in each time-group

d) by Mann-Whitney test

e) 2 animals were lost due to anaesthetic and deaths

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Table 28

Surface Area 1 cm Perimeter of Grid 16 Day Group
(relative to pre-expansion area, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,f]	p value[d] (<)
Pre-expansion	1.00±0	-	3	-
0	1.30±0.02	1.00±0	6	0.01
4	1.23±0.02	0.98±0.02	6	0.01
8	1.27±0.03	1.13±0.03	6	0.01
12	1.38±0.03	1.12±0.02	6	0.01
16	1.41±0.04	1.13±0.02	6	0.01

Table 29

Surface Area 1 cm Perimeter of Grid 32 Day Group
(relative to pre-expansion area, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,g]	p value[d] (<)
Pre-expansion	1.00±0	-	5	-
0	1.35±0.01	1.00±0	10	0.01
8	1.53±0.02	1.07±0.01	10	0.01
16	1.56±0.02	1.07±0.02	10	0.01
24	1.60±0.02	1.11±0.01	10	0.01
32	1.72±0.01	1.10±0.02	10	0.01

b) total of experimentals and controls in each time-group

d) by Mann-Whitney test

f) 6 animals were lost due to anesthetic(4) and post-operative(2) deaths

g) 2 animals were lost due to anaesthetic deaths

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Table 30

Skin Thickness
(mm, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,h]	p value[d] (<)
4	0.317±0.004	0.407±0.003	12	0.01
8	0.329±0.005	0.419±0.005	10	0.01
16	0.354±0.007	0.419±0.006	6	0.01
32	0.398±0.003	0.425±0.004	10	0.01

Table 31

Breaking Strength
(gm, mean ±1 s.e.m.)

Days	Experimental	Control	n[b,h,f]	p value[d] (<)
4	1165±199	1171±142	12	0.63
8	1208±136	1209±103	20	0.93
16	1329±92	1522±79	12	0.14
32	1249±111	1183±72	20	0.47

b) total of experimentals and controls in each time-group

d) by Mann-Whitney test

f) for all groups except the 4-day groups, 2 specimens from each animal were tested

h) 10 animals were lost due to anaesthetic(8) and post-operative(2) deaths

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Table 32

Wet Weight
(gm, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,h]	p value[d] (<)
4	66.5±8.5	72.6±3.6	12	0.17
8	63.1±6.7	53.0±5.6	10	0.20
16	106.8±18.7	65.9±9.6	6	0.08
32	176.9±20.2	81.2±8.4	10	0.01

Table 33

Dry Weight
(gm, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,h]	p value[d] (<)
4	10.9±0.6	13.2±0.6	12	0.04
8	9.5±1.6	7.7±0.6	10	0.39
16	20.1±3.9	10.8±1.3	6	0.05
32	38.8±5.4	18.1±1.1	10	0.01

b) total of experimentals and controls in each time-group

d) by Mann-Whitney test

h) 10 animals were lost due to anesthetic(8) and post-operative(2) deaths

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Table 34

Specific Activity of ^{3}H hydroxyproline
(dpm/mg dry weight, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,h]	p value[d] (<)
4	237±43	258±48	12	0.57
8	854±190	324±75	10	0.01
16	405±53	161±11	6	0.02
32	654±87	269±59	10	0.02

Table 35

Total Hydroxyproline
(mg, mean ± 1 s.e.m.)

Days	Experimental	Control	n[b,h]	p value[d] (<)
4	1.20±0.09	1.31±0.09	12	0.33
8	1.08±0.15	1.26±0.06	10	0.28
16	2.61±0.30	1.43±0.11	6	0.01
32	3.08±0.26	2.02±0.17	10	0.01

b) total of experimentals and controls in each time-group

d) by Mann-Whitney test

h) 10 animals were lost due to anesthetic(8) and post-operative(2) deaths